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ERRATUM.

In Volume XVIII, Number 4, October, 1913, pages 387 and 388 should be reversed. On page 476, line 11, for "experiment D" read "experiment E." On page 477, line 14, for "experiment G" read "experiment H." On page 477, line 32, for "monkey E" read "monkey F."

THE OXYGEN CONTENT OF THE BLOOD IN RABBITS INFECTED WITH PNEUMOCOCCUS.*

By FRANCIS W. PEABODY, M.D.

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It has been shown by experiments *in vitro*[1] that the growth of the pneumococcus on blood decreases the capacity of the blood for combining with oxygen. This effect is seen when the pneumococcus is planted in mixtures of unlaked red corpuscles, but it is more marked in solutions of hemoglobin, and the lowering of the oxygen-combining power is apparently due to the formation of methemoglobin. Even when spectroscopic examination fails to show the absorption bands of methemoglobin, the oxygen-combining power of the hemoglobin is decreased, and spectrophotometric measurements make it certain that methemoglobin is present. The determination of the power of the blood for combining with oxygen is thus a very delicate test for changes taking place in it.

As a result of these experiments *in vitro* it seemed of importance to determine whether similar effects on the blood took place in animals with a pneumococcus septicemia. For this purpose rabbits were used, and the animals were injected with large doses of pneumococcus so as to induce rapidly a profound bacteremia. They were inoculated in the ear vein with the organisms centrifuged out from 200 to 500 cubic centimeters of an eighteen hour bouillon culture. Shortly after inoculation the organisms could be found in smears of the blood, and the number of organisms increased until at death enormous numbers were present. Clinically the most striking symptom was the abnormal respiration. Most of the animals showed a polypnea, but several had a slow, deep respiration, not unlike the typical breathing of diabetic acidosis. As the infection

* Received for publication, March 27, 1913.

[1] Butterfield, E. E., and Peabody, F. W., *Jour. Exper. Med.*, 1913, xvii, 587.

became more marked, a cyanosis of the mucous membranes and conjunctivæ developed and the blood in the ear veins became much darker. Death usually occurred in from four to six hours.

The blood after death varied from a dark reddish brown to a chocolate color. When shaken with air it changed color rather slowly and often failed to become bright red. The coagulation time was increased. Sometimes the blood remained fluid for many hours. The serum was yellowish green and turbid, much as described by Longcope[2] after the growth *in vitro* of pneumococcus in the sera of pneumonia patients. The reaction of the serum to litmus paper was variable. Sometimes it decolorized litmus paper, sometimes it gave a slightly alkaline or an amphoteric reaction, and occasionally it was acid to litmus a short time after death. In general it was, however, much less alkaline to litmus than is normal rabbit serum. The carbon dioxide content of the blood was determined in several animals, and a marked falling was observed as the infection advanced. This is, of course, exactly what occurs in other types of acidosis. Spectroscopic examination of the laked red cells usually showed the absorption bands of oxyhemoglobin or hemoglobin. Only very rarely were the bands of methemoglobin present, although they occasionally appeared after the blood had been kept for some hours *in vitro*.

Observations were made by the Barcroft and Haldane[3] method on the oxygen content of blood from the carotid artery at various times after the animal was inoculated, and on the power of the blood for combining with oxygen at the same time and also immediately after death. The combining power was tested by laking the blood and shaking it for five minutes in air in order to saturate it with oxygen. A source of error exists in these determinations in that the pneumococcus, an organism which uses up oxygen rather rapidly, was present in the blood. There was undoubtedly some loss of oxygen during the analysis of the specimen, but all specimens were analyzed as nearly as possible at the same rate and with the same precautions, and control observations on the rate of destruction of oxygen by the organism in specimens of blood taken

[2] Longcope, W. T., *Jour. Exper. Med.*, 1905, vii, 626.

[3] Barcroft, J., and Haldane, J. S., *Jour. Physiol.*, 1902, xxviii, 232.

after death made it clear that the error was not large enough to affect the general meaning of the results. This source of error was, moreover, finally controlled and in great part eliminated in experiment 8. Blood was taken before inoculation and immediately after death. Both specimens were laked and centrifuged rapidly, so that smears from the blood taken after death contained no pneumococci. This sterile solution of hemoglobin showed a decrease in the oxygen-combining power of over 17 per cent.,—a change exactly similar to that found in the other experiments. It is thus evident that the low oxygen values are not dependent on the presence of organisms in the blood during the analysis, but on a change in the hemoglobin molecule (table I).

The results may be briefly summarized as follows:—The first specimen of blood was taken as a control before inoculation or before the animal showed marked symptoms. It gives the normal oxygen content of the arterial blood for the individual animal, and the content of the blood when saturated with oxygen. Subsequent specimens show a diminution both in the oxygen content and in the oxygen-combining power of the blood. The degree of this diminution is more or less parallel to the severity of the infection, and the fall is progressive until death occurs. At the time when death took place, the oxygen-combining power of the blood was usually between 60 and 75 per cent. of its original value. In some of the experiments the oxygen content fell in approximately the same ratio as the oxygen-combining power. In these, then, the degree of saturation with oxygen remained about normal. In other instances (rabbits 1 and 2), however, the oxygen content fell more rapidly than the combining power, and the arterial blood was much less completely saturated than normally. This low degree of saturation might depend in part on the fact that oxygen is directly used up by the pneumococcus, for it is possible that if the venous blood reached the lungs with an abnormally low oxygen percentage, the time of the passage of the lung capillaries would not be long enough to allow the blood to become completely saturated. On the other hand, as there is a gradual shift in reaction of the serum, and as the hemoglobin molecule is ultimately profoundly changed by the

TABLE I.

The Oxygen Content of the Arterial Blood, and the Oxygen-Combining Capacity of the Blood of Rabbits Infected with Pneumococcus.

Time of inoculation.	Time at which blood was taken.	Per cent. of oxygen in arterial blood.	Per cent. of oxygen in blood when completely saturated.	Remarks.
Rabbit 1. Oct. 22, 1912.	10.30 A.M.	10.81		Control specimen before inoculation.
Inoculated at 10.45 A.M.	12.55 P.M.	1.47		Animal dying. Arterial blood reddish brown.
	1.00 P.M.		7.08	Blood from heart. Heart still beating when chest was opened. After standing 24 hrs. this blood, in strong solution, shows absorption bands of methemoglobin. It decolorizes litmus paper, but does not turn it red.
Rabbit 2. Oct. 24, 1912.	12.30 P.M.	7.12	8.88	No observation made while animal was still normal.
Inoculated at 10.25 A.M.	1.05 P.M.	2.82	6.78	Death at 1.25 P.M. No methemoglobin absorption bands.
Rabbit 3.	2.30 P.M.	11.40	12.51	
Oct. 29, 1912.	4.30 P.M.	10.01	11.20	
Inoculated at 12.45 P.M.	5.00 P.M.		9.79	Blood taken after death. Heart still beating.
Rabbit 4.	11.25 A.M.	14.34	18.59	
Nov. 1, 1912.	12.10 P.M.	13.22	16.37	Dyspnea.
Inoculated at	12.45 P.M.	11.14	12.95	
9.15 A.M.	1.40 P.M.		9.89	Blood taken from heart at death. Heart still beating.
Rabbit 5.	11.40 A.M.	12.11	14.80	
Nov. 7, 1912.	1.00 P.M.	8.02	9.25	
Inoculated at 9.20 A.M.	1.15 P.M.		9.05	Blood taken 5 min. after death. No absorption bands of methemoglobin.
Rabbit 6.	3.25 P.M.		12.21	
Oct. 12, 1912.	4.25 P.M.		10.12	Oxygen content of venous blood, 2.06 per cent. at 4.00 P.M.
Inoculated at 9.00 A.M.	5.20 P.M.		8.81	
	5.40 P.M.		7.71	Rabbit dying.
Rabbit 7. Oct. 8, 1912.	12.30 P.M.		10.28	Control observation before inoculation.
Inoculated at 9.00 P.M.	7.15 P.M.		7.00	Blood taken shortly after death.

growth of the pneumococcus, it is not unlikely that there is a slowing of the reaction between the hemoglobin and oxygen. It is noticeable that when one shakes specimens of infected blood in the air, they appear to become red unusually slowly, but it has not been possible to make any direct observations on the rate with which these bloods take up oxygen.

In pneumococcus septicemia in rabbits, then, the blood is changed so that it takes up less oxygen than normally. This change only begins to appear when the infection is advanced. It is progressive and reaches its maximum at death. Associated with this fall in the oxygen-combining power is a progressive fall in the oxygen content of the arterial blood. The fact that the latter is often relatively greater than the former suggests a slowing or other interference in the reaction between hemoglobin and oxygen. Occasionally specimens of infected blood show the absorption bands of methemoglobin.

The changes in the infected rabbit are exactly analogous to the changes occurring when the pneumococcus grows in blood *in vitro*. In solutions of hemoglobin, practically all the hemoglobin may be converted into methemoglobin. In mixtures of unlaked red blood cells the action is less intense and after twenty-four hours' growth methemoglobin cannot usually be found spectroscopically. There is, however, a profound fall in the oxygen-combining power of the blood, and spectrophotometric measurements show that the change to methemoglobin is taking place. In infected rabbits the change in the blood rarely goes on so far that methemoglobin is distinguishable spectroscopically, for the animals die before this stage is reached. The fall in the oxygen-combining power of blood, however, shows that the hemoglobin is being affected, and this, together with the occasional finding of methemoglobin in the blood from animals, makes it certain that in animals the growth of the pneumococcus produces the same effect on the hemoglobin molecule as it does *in vitro*. It is impossible to decide definitely whether acid production plays a part in the alteration of the hemoglobin, but experiments *in vitro* are contrary to this supposition.

CONCLUSIONS.

In rabbits with a severe pneumococcus bacteremia, the oxygen-combining power of the blood falls progressively up to the time of death. Coincident with this there is an even more marked fall in the oxygen content of the arterial blood.

The changes in the blood in infected animals are analogous to those seen when the pneumococcus is grown on blood *in vitro*. They are due to the conversion of hemoglobin into methemoglobin.

THE OXYGEN CONTENT OF THE BLOOD IN LOBAR PNEUMONIA.*

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For two reasons it seems possible that a study of the oxygen content of the blood in pneumonia might yield results of interest. In the first place, the disease affects primarily the lungs, so that a disturbance of the respiratory exchange of gases might take place. In the second place, the growth of the pneumococcus produces a marked change in the hemoglobin molecule. Butterfield and Peabody[1] have shown that, as a result of its growth on blood *in vitro*, the hemoglobin may be converted into methemoglobin. As yet, however, practically no direct determinations of the oxygen content of the blood in pneumonia have been made. The following study was therefore undertaken to obtain some evidence as to the extent and manner in which the disease affects the oxygen of the blood, and the relation of any changes in the oxygen to the symptoms and course of the disease. Analyses of arterial blood were, of course, greatly to be desired, but in a study on patients it seemed safer to use only venous blood. Since this work was finished, however, Hürter[2] has shown that it is both safe and comparatively easy to take blood from the radial artery. While analyses of peripheral venous blood are not wholly satisfactory, there are certain facts of interest which are brought out by them, and which may serve as a basis for further study.

The method used was that of Barcroft and Haldane,[3] as modified by Brodie.[4] This has been briefly described, together with the

* Received for publication, March 27, 1913.

1 Butterfield, E. E., and Peabody, F. W., *Jour. Exper. Med.*, 1913, xvii, 587.

2 Hürter, *Deutsch. Arch. f. klin. Med.*, 1912, cviii, 1.

3 Barcroft, J., and Haldane, J. S., *Jour. Physiol.*, 1902, xxviii, 232.

4 Brodie, T. G., *Jour. Physiol.*, 1909–10, xxxix, 391.

technique of taking the samples of blood, in a previous paper[5] in which the results of the analyses of the same bloods for carbon dioxide were discussed. It is perhaps well to repeat here that the blood was taken without obstructing the flow in the veins. Nearly all observations were made in duplicate, and few analyses had to be discarded on account of the results failing to agree.

Control observations were made on normal individuals and on patients during convalescence from pneumonia. Twenty-one specimens of blood from seven convalescents and five normal adults showed an oxygen content varying from 9.23 to 15.02 per cent. Of these only three, all from convalescents, were below 10 per cent., and only one, from a normal individual, was above 14 per cent. It is thus important to realize that with subjects at rest and in what may be considered normal conditions, the oxygen content of the peripheral venous blood may vary within rather wide limits. Buckmaster and Gardner[6] report a series of eleven observations made by a different method on their own venous blood, and found the oxygen content to range between 2.63 and 10.8 cubic centimeters per 100 cubic centimeters of blood. In the present study figures as low as some reported by these authors were rarely met with, and then only under pathological conditions.

The material for study consisted of twenty-five cases of lobar pneumonia. In many cases repeated observations were made during the course of the disease and also in convalescence. The total number of determinations was eighty. The results obtained may be conveniently considered in three groups.

The first group of observations was made on cases in which the disease was running an uncomplicated course and in which no abnormality of the oxygen content of the blood was found. This series consists of forty analyses of blood from fifteen cases. In seven cases only a single specimen of blood was taken in the febrile period, but in others as many as six or eight observations were made during the disease and in convalescence. Twenty-seven of the samples were taken during fever and thirteen at a time when

[5] Peabody, F. W., *Jour. Exper. Med.*, 1912, xvi, 701.

[6] Buckmaster, G. A., and Gardner, J. A., *Proc. Roy. Soc.*, 1912, lxxxv, *Ser. B, Biological Sciences*, 56.

the temperature had become normal. The earliest were taken on the third, the latest on the eighteenth day of the disease. In all of the forty observations the oxygen content was between 9.03 and 14.52 per cent., while only four specimens gave more than 14 per cent., and only six gave below 10 per cent. Several cases had a rather higher oxygen content during the febrile period than during the subsequent afebrile period.

This group of cases, which constitutes by far the greater number of uncomplicated instances of pneumonia, shows, then, an oxygen content of the peripheral venous blood which is well within the normal limits. The differences found during the various stages of the disease and the differences between the febrile and convalescent periods do not exceed those found in health. The extent of lung involvement appears to have little influence on the oxygen. In one case the temperature was 103° F. and the rate of respiration thirty-two on the day before the crisis, and the oxygen content was 14.52 per cent. On the following morning the temperature had dropped to 98° F. and the respiration to twenty-four, but the oxygen was 13.55 per cent., in spite of there being no noticeable change in the physical signs in the chest. There is no definite relation between either temperature or respiration and oxygen content, except the rather obvious fact that during the acute stage of the disease the normal oxygen level is associated with a more or less increased respiratory rate. By this increase in the rate of respiration, and perhaps by a more rapid circulation, the organism is thus able to compensate fully for the decrease of respiratory surface caused by the pulmonary exudate. Such compensation is easy, for the lungs have a comparatively large factor of safety. Loewy and von Schrötter[7] state that if one lung were completely shut off by the occlusion of its main bronchus, the venous blood would contain 8 per cent. of oxygen instead of the normal 14 per cent., even if there were no compensation on the part of the respiratory or circulatory systems.

The second group of cases consists of only two patients. Like those in group I, both ran an uneventful course ending in recovery.

[7] Loewy, A., and von Schrötter, H., *Ztschr. f. exper. Path. u. Therap.*, 1905, i, 197.

Both, however, had a diminution in the oxygen content of the blood during the height of the disease. In the first case this diminution was only found once, on the fourth day of illness. The oxygen was then 5.67 per cent., but two days later it was normal and it remained so afterwards. In the second case the low oxygen was found at three separate times during two days. The lowest observation was 2.77 per cent. Before and after this period the oxygen was normal. Both cases were rather more cyanotic than was usual. Blood cultures were negative in both. In both cases the low oxygen figures were associated with values for carbon dioxide which were much higher than normally. The combination of low oxygen and high carbon dioxide suggests some pulmonary or circulatory interference with the normal gaseous exchange. In the second case, however, several observations showed that the blood lacked the power of taking up oxygen normally, even when shaken vigorously in the air. It is thus not improbable that there had been some change in the hemoglobin, and the case is somewhat similar to case 8 which will be discussed in more detail below.

The third group consists of those cases which terminated fatally. Death in pneumonia may result in a variety of ways. It often comes suddenly as the result of cardiac or of respiratory failure. It may be the result of a complication. Not infrequently, however, the clinical history is somewhat as follows: Towards the end of the first week of the disease the patient, who has been doing well, begins to show serious symptoms. The pulse becomes rapid, weak, and perhaps irregular. Respiration is shallow, labored, and rapid. Cyanosis becomes marked and toxemia deepens. After some hours coarse, moist rales begin to appear in the chest. A general pulmonary edema, associated with coarse tracheal rales, develops, the patient becomes increasingly weak and toxic, and dies in twelve to twenty-four hours. This more or less gradual type of death is the one which is most frequently met with, and was present in the majority of the fatal cases in this series. Dochez[8] has analyzed a number of these typical, slowly progressive cases, with a view to finding the actual cause of death. He concludes that in general the patient can withstand the original infection, and that

[8] Dochez, A. R., *Jour. Exper. Med.*, 1912, xvi, 680.

death is due to a spreading of the infection and to the failure of the patient to resist the secondary insult. The spread of the infection may be in either or both of two ways. There may be a further involvement of the lungs, or there may be a spread to the blood and the development of an intense bacteremia. The degree to which the infection may spread in the lungs or in the blood before death ensues depends, of course, on the resistance of the patient.

Eleven fatal cases are reported here. Ten of these were of the slowly progressive type just described. One case (No. 11) died very suddenly, apparently of respiratory failure. There was no pulmonary edema and the heart sounds were audible for three minutes after respiration stopped. Blood cultures were made in ten cases. In nine of these they were positive, and bacterial counts by Dochez (table I) show that there was often a bacteremia of progressively increasing intensity. The number of organisms was frequently over 1,000 per cubic centimeter of blood at the time of death. In one case (No. 8) blood cultures were negative, even on the day of death, but the physical signs and autopsy showed a rapidly spreading pulmonary lesion.

Observations on the oxygen content of the blood during life were made in ten of the eleven fatal cases. In the one case that died suddenly (No. 11), the oxygen content of the blood was normal two hours before death. In one case (No. 10) the last specimen of blood was taken twelve hours before death and showed a normal oxygen content. In the other eight cases observations were made one or more times during the final stage of the disease and in every case the oxygen content was below normal. Moreover, these cases show (table I) that during the terminal stage the fall of the oxygen content of the blood is, roughly, a progressive one. Thus samples of blood taken from different cases ten, five and one half, and three hours before death contained 6.89, 8.40, and 8.12 per cent. of oxygen, respectively. Others taken two hours, one hour, and thirty minutes before death contained 4.68, 2.30, and 2.07 per cent. of oxygen. A specimen taken five minutes after death contained no oxygen. In several cases (Nos. 3, 4, 5, and 8) the oxygen content of the blood had previously been found to be normal, and the fall in the oxygen content accompanied the develop-

TABLE I.

The Oxygen Content and the Oxygen-Combining Power of the Blood in the Fatal Cases of Pneumonia.

No. of case.	Date (1912).	Per cent. of oxygen content of blood.	Per cent. of oxygen-combining power of blood.	Blood culture.[9]	Remarks.
1	Mar. 29, 10 A.M.	7.80		4,500 colonies per 1 c.c. of blood	Marked cyanosis. Many coarse rales.
T. W. 525	10 P.M.	8.40	16.35		
	Mar. 30		7.87		Blood taken 10 min. after death.
2 H. L. 453	Jan. 31	2.30			Cyanosis. Edema of lungs. Blood taken 1 hr. before death.
3	Mar. 29	9.03	16.02	Positive on Mar. 28.	
M. M. 533	Apr. 2			15,000 colonies per 1 c.c. of blood	
	Apr. 3	2.07	13.23		Cyanosis. Many coarse rales. Blood taken 30 min. before death. Hemoglobin, 96 per cent.
4	Jan. 15	10.97		Positive on Jan. 14	
T. C. 423	Jan. 16	4.68			Many coarse rales. Cyanosis not marked. Blood taken 2 hrs. before death.
5	Jan. 25	10.41			
J. McL. 438	Jan. 26	6.89		100 colonies per 1 loop of blood	Marked cyanosis and edema. Blood taken 10 hrs. before death.
6	Apr. 9	8.27		Negative	
J. R.	Apr. 11	6.86			Slightly cyanotic.
547	Apr. 13	3.94	16.29	4 colonies per 1 c.c. of blood	Hemoglobin, 88 per cent. Cyanosis marked.
	Apr. 15	8.47	16.71	21 colonies per 1 c.c. of blood	Hemoglobin, 88 per cent. Cyanosis less marked.
	Apr. 16		13.95		Died suddenly. Blood taken 10 min. after death. Serum reacts alkaline to litmus paper.
7	Apr. 6	3.61	18.59	Negative on Apr. 5	Cyanosis very marked.
N. A.	Apr. 8	4.44	17.50	Negative on Apr. 9	
544	Apr. 12		15.09	2,500 colonies per 1 c.c. of blood 1 hr. after death	Pulse weak. Many rales in chest. Blood taken 2 min. after death.

[9] The blood cultures were made by Dr. Dochez.

TABLE I.—*Continued.*

No. of case.	Date (1912).	Per cent. of oxygen content of blood.	Per cent. of oxygen-combining power of blood.	Blood culture.	Remarks.
8	Feb. 2	10.81		Negative	
J. D.	Feb. 3	11.98			
460	Feb. 4, 4.30 A.M.	6.84	15.07		Coarse bubbling rales throughout chest.
	9.30 A.M.	9.07			
	2.30 P.M.	9.71			
	9.45 P.M.	12.09			Chest much more clear. Few rales. Cyanosis much less.
	Feb. 5 10.00 A.M.	8.12		Negative	Many rales in chest.
	1.00 P.M.	0.00	15.05		Blood taken immediately after death. Autopsy showed almost complete solidification of right lung,—apparently a spreading lesion.
9	Mar. 4		16.38	125 colonies per 1 c.c. of blood	Blood taken 2 min. after death.
I. J. 535					
10	Feb. 12	7.78		Negative	Cyanotic and exhausted after a long walk to the hospital.
W. H. 472	Feb. 13	12.64			
	Feb. 14	11.93		Positive	
	Feb. 15	12.71		Positive	Blood taken 12 hrs. before death. Pulmonary edema.
			16.84	1,000 colonies per 1 c.c. of blood	Blood taken 8 min. after death.
11	Feb. 21	13.28	19.35	150 colonies per 1 loop of blood	Blood taken 2 hrs. before death. No pulmonary edema. Respirations very rapid, but became slow and stertorous before death. Heart sounds audible for 3 min. after respiration stopped.
W. H. 480					
					Average capacity of 6 normal individuals and 6 convalescents, 19.57 per cent.

ment of the severe terminal symptoms and of the bacteremia, or, as in case 8, the rapid spread of the process in the lung. Thus in almost all cases of pneumonia running an uneventful course, the oxygen content of the blood remains within normal limits, but in

the majority of fatal cases, the terminal stage is marked by a progressive decrease in the oxygen content of the blood.

Since the pneumococcus shows an intense avidity for oxygen, it was possible that the mere presence of the organism in the blood was sufficient to explain the decrease in the oxygen content of the blood. It was therefore important to determine whether there was any actual change in the hemoglobin molecule which interfered with its taking up oxygen normally. For this purpose samples of blood were shaken in air until saturated with oxygen, and then the total combined oxygen was determined. Six specimens of blood from five convalescent pneumonia cases and one normal individual gave an average oxygen capacity of 19.57 per cent. In eight of the fatal pneumonia cases, similar observations were made. Case 11, which, as has been said, died very suddenly, had a normal oxygen content of the blood two hours before death and showed at the same time a normal oxygen-combining power. On the other hand, all the cases which had a low oxygen content showed also a low oxygen-combining power in specimens of blood taken just before or, as nearly as possible, at the time of death. The progressive nature of this fall in the oxygen-combining power of the blood was shown in four out of five cases, in which more than one observation was made during the terminal stage. In the fifth case the blood had as low a capacity twenty-four hours before death as it did at death. The amount of oxygen given off by fully saturated samples of blood taken at death varied considerably from case to case. The highest was 16.84 per cent., the lowest was 7.87 per cent. Four were between 13.23 and 15.09 per cent. In general, then, although the fall in the oxygen-combining power of the blood is slightly less marked than is the fall in the actual oxygen content of the venous blood, the two run parallel to one another.

The blood after death was very dark and often of a brownish tint. It coagulated much more slowly than normally. The serum was alkaline to litmus in the only case tested. Spectroscopic examination in several cases showed only the absorption bands of oxyhemoglobin or hemoglobin. Methemoglobin was not found. Hemoglobin estimations on several cases ruled out the possibility of the low oxygen-combining power being due to anemia.

The explanation of the striking changes shown in the oxygen content and in the oxygen-combining power of the blood in the terminal stage of fatal cases of pneumonia is to be found by turning to experimental studies. Butterfield and Peabody[10] have shown that when the pneumococcus grows in a mixture containing red blood cells, there is a gradual conversion of the hemoglobin into methemoglobin, and coincident with this a decrease in the extent to which the blood can take up oxygen. There is, moreover, a marked fall in the oxygen-combining power of such mixtures, even when no methemoglobin absorption bands can be distinguished spectroscopically. Peabody[11] has also found that similar changes take place in rabbits with a severe pneumococcic bacteremia. When the infection in rabbits becomes advanced, there is a gradual fall in the oxygen content of the arterial blood and also in the oxygen-combining power of the blood. The fall both in the oxygen content of the arterial blood and in the oxygen-combining power is a gradually progressive one up to the time of death. At the time of death the combining power is usually only between 60 and 75 per cent. of its original value. In some animals the oxygen content of the blood falls more rapidly than the oxygen-combining power, so that the arterial blood is less completely saturated with oxygen than under normal conditions. The suggestion was thus made that, besides a fall in the actual combining power of the blood, there is also a slowing in the rate of the reaction.

Conditions similar to those produced experimentally in rabbits are found in the fatal cases in man. In both there is a progressive decrease in the oxygen content of the blood. This has only been demonstrated for the venous blood of man, but it occurs in the arterial blood of rabbits and probably also in the arterial blood of man. In man and in the rabbit there is also a progressive diminution in the oxygen-combining power of the blood, reaching its minimum at the time of death. In the experimental infections and usually in the fatal cases in man there is a severe bacteremia. The conditions in man and in the rabbit are so similar to those in the test-tube that it seems logical to explain all three in the same way

[10] Butterfield, E. E., and Peabody, F. W., *loc. cit.*

[11] Peabody, F. W., *Jour. Exper. Med.*, 1913, xviii, 1.

and to account for the changes in the blood by the growth of the pneumococcus. In solutions of laked hemoglobin the pneumococcus produces amounts of methemoglobin that are easily found with the spectroscope. In mixtures of unlaked red cells the change goes on much more slowly and is less complete. In the human body and in experimental animals death occurs before enough methemoglobin has been formed to be visible spectroscopically, but the falling off of the oxygen-combining power of the blood indicates that such a change is probably taking place. Case 8 never had any demonstrable bacteremia, but it has been shown that if a bouillon culture of the pneumococcus be passed through a Berkefeld filter, the filtrate will have the same effect on blood as does the organism itself. It may thus be assumed, with comparative certainty, that the changes in the blood in this case are due to the passage into the blood stream of the products of the growth of the organism in the lungs. Case 11, the only fatal case not showing changes in the oxygen of the blood, died suddenly. It is possible that had he lived longer, the organisms would have developed and produced the characteristic changes in the blood.

The progressive fall in the oxygen content and in the oxygen-combining capacity of the blood in fatal cases of pneumonia is thus undoubtedly the direct result of the growth of the pneumococcus. An interference with the respiratory exchange of gases in the lungs, or a circulatory effect could not explain the conditions, for the low oxygen was associated with a low carbon dioxide content of the blood,[12] and any condition which interfered with the taking up of oxygen would also interfere with the excretion of carbon dioxide. Moreover, an abnormal respiratory exchange would not affect the oxygen-combining power of the blood. The same change is also present in the arterial blood of rabbits infected with the pneumococcus, and in these animals the lungs are not involved. In how far the changes produced in the blood are the actual cause of death, it is impossible to say, but that they are a factor, and probably an important one, can hardly be denied. The terminal symptoms,—toxemia, cardiac failure, polypnea, pulmonary edema, and cyanosis,—run parallel to the changes in the blood and may well

[12] Peabody, F. W., *loc. cit.*, 1912, xvi, 701.

be due in part to a deficient oxygenation dependent on these changes. It is interesting that in case 8 the temporary rise in the oxygen values was associated with a marked clinical improvement. While not attempting to assign a rôle that is all important to the changes in the blood, it would seem correct to consider that in many cases of pneumonia the terminal symptoms and death are in part dependent on the changes produced in the hemoglobin molecule by the growth of the pneumococcus.

CONCLUSIONS.

In most cases of uncomplicated lobar pneumonia the decrease of respiratory surface is completely compensated for, and the oxygen content of the blood is within normal limits. Occasional cases of uncomplicated pneumonia have an oxygen content of the venous blood which is below normal. In the two cases reported here, this was associated with a carbon dioxide content of the blood which was higher than normally, and the condition was apparently due to an interference with the respiratory exchange of gases.

In the terminal stage of the fatal cases of pneumonia in which death does not occur with great suddenness, there is often a progressive diminution in the oxygen content of the blood. Synchronous with this is a progressive decrease in the oxygen-combining capacity of the blood. These changes are usually seen in patients in whom an intense bacteremia has developed and are analogous to those found in the arterial blood of infected rabbits, and to those resulting from the growth of the pneumococcus in blood *in vitro*. In all three conditions there is probably a change of oxyhemoglobin to methemoglobin. This change of the hemoglobin molecule, so that it no longer takes up and gives off oxygen readily, is probably a factor in the immediate cause of death in many cases of pneumonia.

CONCERNING AGGLUTININS FOR TREPONEMA PALLIDUM.*

By JOHN A. KOLMER, M.D.

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The relation of *Treponema pallidum* to syphilis has been conclusively proven by Noguchi, who reproduced lesions of syphilis in the testicles of rabbits and in the eyebrows of monkeys by implantation of a pure culture of *Treponema pallidum* isolated from a syphiloma of a testicle of the rabbit due to inoculation with material from a human lesion. Schereschewsky,[1] in 1909, was probably the first to show that spirochetes may be grown artificially, although in 1907 Levaditi, Yamanouchi, and others found that *Treponema pallidum* could be kept alive in a celloidin sac containing monkey serum, placed in the peritoneal cavity of a monkey. Later, in 1909 and 1910, Mühlens[2] and Hoffmann[3] claim to have grown *Treponema pallidum* in Schereschewsky's gelatinized horse serum medium and to have purified a culture in horse serum agar. Early attempts at inoculation were unsuccessful, although at a later period Hoffmann reported a successful inoculation. The lesion, however, was doubtful, the culture grew without the presence of fresh tissue and produced a strong putrefactive odor, thus resembling the morphological and biological characters of *Treponema microdentium* as described by Noguchi. In 1910 Bruckner and Galasesco[4] reported the successful inoculation of rabbits with impure cultures of *Treponema pallidum* grown in gelatinized ascitic fluid in which the original syphilitic tissue was still present. Noguchi[5] has also cultivated

* Received for publication, April 2, 1913.

[1] Schereschewsky, J., *Deutsch. med. Wchnschr.*, 1909, xxxv, 835, 1260, 1652.

[2] Mühlens, P., *Deutsch. med. Wchnschr.*, 1909, xxxv, 1261; *Klin. Jahrb.*, 1910, xxiii, 339.

[3] Hoffmann, *Ztschr. f. Hyg. u. Infectionskrankh.*, 1911, lxviii, 27.

[4] Bruckner, I., and Galasesco, P., *Compt. rend. Soc. de biol.*, 1910, lxviii, 684.

[5] Noguchi, H., *Jour. Exper. Med.*, 1911, xiv, 99; 1912, xv, 90, 201.

Treponema pallidum directly from human lesions, and the definite characteristics of his cultures as compared with pure cultures of other spirochetes isolated by him, together with the successful inoculation of monkeys and rabbits, and confirmatory evidence afforded by specific complement fixation and luetin reactions, prove conclusively the etiological relation of *Treponema pallidum* to syphilis.

Until the present time studies of the antibodies of *Treponema pallidum* have been chiefly confined to the syphilitic antibody concerned in the complement fixation reaction of syphilis. The object of the present communication is to record briefly a study of agglutinins for *Treponema pallidum* (Noguchi) in experimental and in human syphilis.

METHOD.

Culture.—The culture of *Treponema pallidum* used in this study was obtained from Dr. Noguchi. The spirochete grows well in ascitic agar with rabbit kidney and fulfills all the cultural and morphological characteristics described by him. It is readily grown in a fluid medium of horse serum bouillon with kidney extract and this medium was selected for the agglutination reactions. In this medium rich cultures of motile spirochetes are secured in two weeks, showing twenty to forty organisms per field when examined by dark-field illumination. In no instance was coagulation of the horse serum or ascitic fluid found to occur. Motility is best seen in young fluid medium cultures, and practically all phases of longitudinal division have been noted.

Immune Sera.—Injections of ascitic agar and serum bouillon cultures into the testicle and scrotal tissue of rabbits failed to develop a syphilitic lesion. The culture, however, was isolated in October, 1910, and it is probable that *Treponema pallidum* quickly loses its pathogenicity in artificial culture media. The rabbits, however, as will be seen later, showed the presence of agglutinin and an amboceptor.

Another series of rabbits was immunized by giving intravenously increasing doses of young unheated fluid cultures. At the end of two months ten cubic centimeters were given at one injection, and in no instance have the animals shown ill effects. The sera of these animals were found to have a high agglutinin content.

Human Sera.—Nineteen sera and one cerebrospinal fluid from patients giving a history of syphilis and yielding a positive Wassermann reaction were studied; also ten sera and two cerebrospinal fluids giving a negative history and negative Wassermann reaction. In no instance were agglutinins found in as low dilutions as 1 : 20.

Technique.—The macroscopic test was employed. Cultures of *Treponema pallidum* were grown in serum bouillon for four weeks, thoroughly shaken, and centrifuged for five minutes. The result was a culture of good density showing twenty to forty spirochetes per field and free from clumping. One cubic centimeter was used in each test, the serum being diluted in one cubic centimeter of sterile saline solution, the total volume, therefore, being two cubic centimeters. By using this amount of culture a sufficient number of spirochetes were present to yield unmistakable agglutination in positive reactions.

All the sera were unheated in dilutions varying from 1 : 20 to 1 : 1,280. After reactions were set up the tubes were incubated for two hours at 37° C. and then placed in the refrigerator over night, final readings being made at the end of twenty-four hours.

In every instance a culture control and controls with normal rabbit and human sera were set up at the same time and in no instance were there any evidences of agglutination or sedimentation.

In most of the tests a drop of the supernatant fluid was examined by dark-field illumination at the time the results were read, and in every instance the controls and negative reactions showed the presence of a large number of spirochetes, a few still motile, and all free from clumping. In a few instances two or three spirochetes were found in clumps, but there was no sedimentation and this was not considered as an evidence of agglutination. In all positive reactions a drop of the sediment examined by dark-field illumination showed the presence of large clumps of spirochetes which were in most instances non-motile, although a few motile organisms were to be found at the edge of a clump, or entirely free. In the higher dilutions where agglutination was incomplete, free active spirochetes were found in the supernatant fluid and clumps in the sediment.

One of the human sera was unfortunately infected with *Bacillus*

subtilis, and a small amount of sediment was readily noted in the test-tubes. An examination by dark-field illumination quickly showed motile non-clumped spirochetes and revealed the cause of the pseudoreaction.

The agglutinated spirochetes stained well with the Giemsa stain and did not show any distinct evidence of disintegration.

RESULTS.

(*a*). *Rabbit Serum.*—Two rabbits were given injections into the testicular tissue of 0.1 of a cubic centimeter of a culture removed from the vicinity of the piece of kidney in a three weeks old ascitic agar culture rich in spirochetes. The animals did not develop any evidence of syphilis. The serum of one showed agglutination in dilution of 1:20 four weeks after inoculation, the second rabbit agglutinating in dilutions up to 1:40.

Three rabbits received the same dose into the testicular tissues at the same time, and at the end of four weeks another dose of one cubic centimeter of a rich serum bouillon culture subcutaneously in the scrotal tissues. Four weeks later the serum was tested and found to agglutinate in dilutions varying from 1:160 to 1:320. None of the rabbits developed lesions of syphilis, one showing a firm nodule at the site of inoculation but free of spirochetes and probably due to non-specific inflammation.

Of additional interest are the results of complement fixation reactions, especially with spirochetal culture antigens. Further study[6] of this subject has been made, but it will suffice to include here a brief record of results with the sera tested for agglutinin. Salt solution and alcoholic extracts of *Treponema pallidum* were used; also, for controls, extracts of sterile media and media inoculated with other organisms. Specific reactions were observed especially with the salt solution extract of the pallida, the spirochetes having been grown in fluid culture and washed free of all media before extraction. Noguchi[7] demonstrated the presence of specific amboceptor in the sera of rabbits immunized with a culture, using a salt

[6] Kolmer, J. A., and Williams, W. W., A Study of Complement Fixation in Syphilis with *Treponema pallidum* Antigen, *Jour. Med. Research,* 1913 (in press).

[7] Noguchi, H., *Jour. Am. Med. Assn.,* 1912, lviii, 1163.

solution extract of the pallidum culture in ascitic agar as antigen. Rabbits suffering with acute syphilitic orchitis yielded negative reactions. Craig and Nichols[8] using alcoholic extracts of pallidum culture also secured negative reactions.

The Wassermann reactions were performed with four different lipoidal extracts. With rabbit sera the results must be regarded as doubtful because the positive results cannot be regarded as specific reactions, for, as will be seen later, normal rabbit serum has been found to yield positive Wassermann reactions under certain conditions.

The following table gives the results of the complement fixation and agglutination reactions.

TABLE I.

No.	Dose of culture.	Complement fixation reaction.		Agglutination reactions.							
		Wassermann reaction.	Spirochete reaction.	1:20	1:30	1:40	1:60	1:80	1:160	1:320	1:640
14	0.1 c.c. into testicle	−	+++	+	−	−	−	−	−	−	−
15	0.1 c.c. into testicle	−	++	+	+	+	−	−	−	−	−
16	0.1 c.c. into testicle 1.0 c.c. three weeks later into scrotal tissues	±	+++	+	+	+	+	+	+	+	−
17		−	+++	+	+	+	+	+	+	+	−
18		−	+++	+	+	+	+	+	±	−	−

Three rabbits were gradually immunized by the intravenous administration of increasing doses of unheated rich serum bouillon cultures until ten cubic centimeters could be given at one time. All the animals withstood the reactions well. The sera showed agglutination in high dilutions.

TABLE II.

No.	Dose of culture.	Complement fixation reaction.		Agglutination reactions.								
		Wassermann reaction.	Spirochete reaction.	1:20	1:30	1:40	1:80	1:160	1:320	1:480	1:640	1:1280
34	Intravenous	−	+++	+	+	+	+	+	+	−	−	−
35	Intravenous	−	++++	+	+	+	+	+	+	+	+	+
36	Intravenous	++	++++	+	+	+	+	+	+	+	+	+

[8] Craig, C. F., and Nichols, H. J., *Jour. Exper. Med.*, 1912, xvi, 336.

Controls.—The fresh sera of four rabbits did not show any evidence of agglutination in dilution as low as 1 : 20. All four yielded negative complement fixation reactions with spirochetal antigens. One yielded a positive Wassermann reaction and after an examination of a large number of normal rabbits we have found a certain proportion to yield a positive Wassermann reaction with certain antigens.[9]

(*b*) *Human Sera.*—Nineteen sera from cases of secondary and tertiary syphilis yielded negative agglutination reactions. All the sera gave a positive Wassermann reaction. The reactions with the spirochetal antigens were in most cases weaker and more inconstant.

TABLE III.

Serum.	Diagnosis.	Complement fixation reaction.		Agglutination reaction in dilutions of 1:20 to 1:640.
		Wassermann reaction.	Spirochete antigen.	
R. L.	Chancre 5 yrs. ago; some treatment	++++	−	All negative.
F. B.	Secondary syphilis	+++	++	All negative.
T. D.	Secondary syphilis	++	−	All negative.
F. D.	Tertiary syphilis	++	++	All negative.
I. G.	Tertiary syphilis	++++	++	All negative.
I. T.	Chancre 3 mos. ago	++++	−	All negative.
W. B.	Secondary syphilis	++	−	All negative.
J. B.	Secondary syphilis	+++	−	All negative.
W. T.	Secondary syphilis	++	+	All negative.
J. R.	Tertiary syphilis	++++	+++	All negative.
C. B.	Tertiary syphilis	++++	+	All negative.
M. T.	Tertiary syphilis	+	−	All negative.
R. W.	Secondary syphilis	++++	−	All negative.
G. W.	Tertiary syphilis	+	+	All negative.
T. C.	Tertiary syphilis	++	−	All negative.

[9] Kolmer, J. A., and Casselman, A. J., Concerning the Wassermann Reaction with Normal Rabbit Serum, *Jour. Med. Research*, 1913 (in press).

TABLE III.—*Continued.*

Serum.	Diagnosis.	Complement fixation reaction.		Agglutination reaction in dilutions of 1:20 to 1:640.
		Wassermann reaction.	Spirochete antigen.	
C. N.	Tertiary syphilis	++++	++	All negative.
C. S.	Tertiary syphilis	+++	++	All negative.
W. H.	Suspicious sore	−	−	All negative.
B. T.	Tertiary syphilis	+++	+	All negative.

Ten sera from persons giving a negative history, negative complement fixation reactions, and free of all clinical evidence of syphilis were also tested and yielded negative agglutination reactions in dilutions of 1 : 20 to 1 : 640.

(*c*) *Cerebrospinal Fluid.*—Three specimens were examined. One yielded a positive Wassermann reaction but none were tested with the spirochetal antigens. None showed the presence of a demonstrable quantity of agglutinin.

TABLE IV.

Spinal fluid.	Diagnosis.	Wassermann reaction.	Agglutination reactions in dilutions of 1:20 to 1:640.
J. Q.	Disseminated cerebrospinal sclerosis	++++	All negative.
J. B.	Pseudobulbar palsy	−	All negative.
M. B.	Hemiplegia	−	All negative.

CONCLUSIONS.

1. There is no demonstrable amount of agglutinin for *Treponema pallidum* (Noguchi) in normal human and normal rabbit serum in dilutions as low as 1 : 20.

2. Agglutinins for *Treponema pallidum* are readily produced in young rabbits by the administration of pure cultures of living spirochetes.

3. There is no appreciable amount of agglutinin for *Treponema pallidum* culture used in the sera of secondary and tertiary syphilis or in the cerebrospinal fluid of tertiary syphilis in dilutions of 1 : 20 to 1 : 640.

I wish to express my appreciation of the aid given me by Dr. William Whitridge Williams and Dr. Ernest E. Laubaugh.

INTESTINAL OBSTRUCTION IN THE RABBIT. II.*

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In an earlier paper[1] concerning intestinal obstruction in the rabbit, we have emphasized the fact that obstruction by ligature of the duodenum is more quickly fatal than obstruction of the pylorus or of the ileum. It was further stated as our belief that death in duodenal obstruction is due to the resorption of a toxic secretion of the duodenal mucosa, a secretion which is rapidly poured out into the lumen of the intestine after obstruction and was shown to produce fatal results in healthy rabbits if injected intraperitoneally in appropriate amounts. The explanation for the delayed death in low obstruction, it was suggested, might lie in the theory of Draper that for a time, at least, this duodenal secretion is neutralized by the secretion of the mucosa of the ileum and jejunum. By offering such an explanation we intimated as our tentative belief that in both high and low obstruction of the small intestine the cause of death lay in this toxic duodenal secretion. While this was not directly stated, it was our belief that this was the fact, except in those cases in which bacterial invasion of the injured mucosa and of the peritoneum could be demonstrated.

Before such a theory could be established, the toxicity of the secretion from isolated loops of the ileum and jejunum must be compared with that from the duodenum. Even in fasting animals, the duodenal secretion is poured out rapidly and apparently continuously, so that within four or five hours after obstruction, the duodenum above the lower ligature is tense with a clear, slightly straw colored, mucin-containing, alkaline solution. At this time, one obtains about one cubic centimeter of fluid per each linear centimeter of the obstructed loop. To determine whether a similar

* Received for publication, April 14, 1913.

[1] Bunting, C. H., and Jones, A. P., *Jour. Exper. Med.*, 1913, xvii, 192.

phenomenon occurred lower down in the small intestine, a small series of fasting animals was operated upon. Loops of intestine of sufficient length to avoid the danger of strangulation were isolated by obstructing the lumen at either end by coarse soft ligatures, applied so as to do the least possible injury to mucosa and blood vessels.

In two animals (rabbits 92 and 93 of the series) such loops of the ileum were made under ether anesthesia after the animals had fasted thirty-six hours. In rabbit 92 the lower ligature was placed just above the ileocecal juncture; the upper, thirty-four centimeters above. In rabbit 93 the lower ligature was similarly placed, and the upper was twenty-eight centimeters above. Four hours after the first operation, the animals were again etherized, the abdomen was opened, and the condition of the intestine noted. In both cases the isolated portion of the intestine was found to be flat and empty. The duodenum, however, was well filled with fluid which had begun to flow downward into the jejunum. Both animals died between thirty-six and forty-eight hours after the primary operation, and in both the post-mortem examination showed the intestine above the upper ligature well filled with secretion, but the section between the ligatures was as empty of fluid as at the time of operation. There had been no secretion into it.

In two other animals (rabbits 94 and 95), with similar treatment and technique, jejunal loops instead of ileal were isolated. In rabbit 94 a loop twenty-six centimeters in length, with the upper ligature fifty-six centimeters from the pylorus, was tied off. In rabbit 95 the upper ligature was placed thirty-two centimeters from the pylorus, just beyond the entrance of the pancreatic duct, while the second was placed forty-five centimeters further down. Rabbit 94 was subjected to a second operation four hours later, and it was found that the duodenum was becoming tense with fluid, while the segment between the ligatures was flat. At autopsy on the two animals the same condition was found as in the animals with ileal loops. There was no secretion whatever into the jejunum, but the duodenal segment above was tense with fluid.

It seemed probable from the two sets of animals, that in the fasting rabbit the duodenum is the only part of the small intestine

which secretes actively into the lumen. It was necessary, however, to meet the objection that peristaltic waves from above, or gastric or hepatic secretion, might stimulate the duodenal mucosa to secretion, while the upper ligature prevented the passage of such stimuli to the lower segment, and thus inhibited secretion. The rabbit is a favorable animal for the determination of this point, because of the length of its duodenum, which may be held to extend to the entrance of the pancreatic duct, a distance of from twenty-five to thirty centimeters from the pylorus.

In rabbit 96, therefore, at the first operation a series of ligatures was placed about the intestine. The first was seven centimeters below the pylorus; the second was at the entrance of the pancreatic duct, and so tied as to occlude both the duct and the intestine. The third ligature was placed twenty-one centimeters further down, the fourth thirty-two centimeters, the fifth thirty-eight centimeters, and the sixth thirty-eight centimeters further. Four hours after operation the first loop was tense with secretion, the second well filled, and all others empty. At autopsy, within twenty-four hours after the operation, the same condition was found, the secretion stopping short at the ligature at the pancreatic duct level.

In rabbit 105 the same procedure was employed, with the exception that the first ligature occluded the pylorus and bile duct, the second was ten centimeters below this, the third was so tied as to occlude pancreatic duct and intestine, and the lower ligatures were spaced at a distance of about fifteen centimeters. At the post-mortem examination, twenty hours after the operation, the two duodenal loops were found widely distended with fluid, while all the others were flat and empty.

It is evident from this that a tight ligature placed about the duodenum does not interfere with the secretion by the segment below the ligature, and we seem justified then in our conclusion that in the fasting rabbit the only section of the small intestine which secretes into the lumen is that lying between the pylorus and the entrance of the pancreatic duct.

This upper segment of the small intestine apparently differs from that lying immediately below the entrance of the pancreatic duct in but one particular,—the presence of the glands of Brünner.

In the rabbit, as shown by section of several duodena, these glands are distributed for a distance of from twenty-five to thirty centimeters from the pylorus, a distance quite coincident with the actively secreting part of the intestine, as shown by our experiments. Consequently it seems justifiable to conclude that the glands of Brünner furnish the secretion we have found in these cases.

As our experiments have been conducted only on the rabbit, the evidence offered can at present be applied only to that species of animal. But from this series, as well as from other experiments, the results of which were reported in the earlier paper, we feel assured that in the fasting rabbit the duodenal mucosa alone secretes, that this secretion comes from the glands of Brünner, and, being toxic, is responsible for the death of the animal with obstruction of the small intestine, at whatever level the obstruction be made.

A STUDY OF THE MECHANICAL OBSTRUCTION TO THE CIRCULATION OF THE KIDNEY PRODUCED BY EXPERIMENTAL ACUTE TOXIC NEPHROPATHY.*

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Plate I.

Part I.

At the suggestion of Dr. W. T. Councilman, I undertook to study the influence of disease on the circulation of various organs, as indicated by the method of perfusion. Perfusion of dead organs has been made by Brodie (1), Sollmann (2), Loebell and Bidder (3), and Langendorff (4), but, as far as I could ascertain, no one had previously attempted to employ perfusion in the study of diseased organs.

The first experiment attempted was the perfusion of a normal rabbit's lung with an 0.85 per cent. solution of sodium chloride warmed to 40° C., and under constant pressure of 100 millimeters of mercury. Difficulties in the perfusion were at once met with, which were brought about by edema of the lung tissues. As this was thought to be due to the liquid used, a fluid was sought that would not give rise to edema. The investigators mentioned above had used many fluids for perfusion, none of which was entirely satisfactory. It was also decided to work first with the kidney, because of the studies already done on perfusion of this organ by Brodie and Sollman, and the work in experimental nephropathy, particularly that of Christian (5) and of Pearce (6). Hence, an attempt was made to find a fluid that would not give rise to an edema in the kidney tissues.

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METHOD.

Pieces of rabbit kidney were weighed and put into bottles containing different fluids in which they were left for one hour. They were then taken out and weighed again with the following results:

TABLE I.

Fluid.	Weight before, in gm.	Time in fluid in hrs.	Weight after, in gm.	Difference.	Per cent. of increase in weight.
1. 1 per cent. salt solution.....	3.350	1	3.590	0.240	7.0
2. 9 per cent. salt solution.....	2.555	1	2.675	0.125	4.9
3. 1 per cent. salt +2 per cent. albumen solution.....	2.050	1	2.160	0.110	5.0
4. Locke's solution.....	2.655	1	2.735	0.080	3.0
5. Ringer-Locke solution.....	2.255	1	2.405	0.150	10.0
6. Ringer-Langendorff solution..	2.930	1	3.170	0.140	4.0
7. Rabbit urine.....	1.460	1	1.600	0.140	8.0
8. Rabbit's own urine.....	1.000	1	1.700	0.700	70.0
9. Pig serum.....	1.770	1	1.870	0.100	6.0
10. Animal's own serum.....	1.320	1	1.350	0.300	2.0

This, of course, was a gross method; but at the same time it sufficed to show that none of the fluids employed was exempt from giving rise to an edema. Therefore other fluids were sought. The fluid described by Adler (7) and found by him to be the most suitable medium for continuing the action of the heart was tried, and more care was taken to learn what degree of edema the fluids produced. Kidneys from freshly killed rabbits were used. The kidney was cut into six pieces and to eliminate the possibility of error in the determination of the amount of edema, the sections were dealt with as follows: Each section was dropped into a vessel containing the solution to be tested. It was then taken out and hung in a moist chamber. The excess of fluid was thus allowed to run off. At the end of a certain time the drop hanging from the end of the section was blotted off. The section was then weighed in a chemical balance and put into a bottle containing the fluid, and the bottle was placed in an incubator kept at 37° C. for one hour. The section was then taken out and hung again in the moist chamber for a length of time corresponding to the time the section remained in the chamber before being weighed. The drop hanging to the end of the section was blotted off and it was weighed again, the difference in weight being noted.

As table I shows, the rabbit's own serum was the fluid that gave rise to the least edema in the first series of experiments. It was consequently decided that in carrying on further tests it was advisable to use this fluid as a control. Therefore, while one of the rabbit's kidneys, cut into six pieces, was placed in Adler's solution, the other kidney, cut in a similar fashion, was placed in bottles containing the rabbit's own serum.

The following tables give the results obtained.

TABLE II.

In this series the sections were put in blood serum.

No. of section.	Time in moist chamber in min.	Weight before.	Time in serum in hrs.	Time in moist chamber in min.	Weight after.	Difference in weight.	Per cent. of increase in weight.
1	15	0.655	1	15	0.695	0.040	
2	27	0.428	1	25	0.468	0.040	
3	32	0.593	1	28	0.612	0.019	
4	37	0.610	1	37	0.646	0.036	
5	40	0.481	1	41	0.500	0.019	
6	45	0.382	1	47	0.407	0.015	
Total weight = 3.149				Total increase = 0.169			5

TABLE III.

In this series Adler's solution was used.

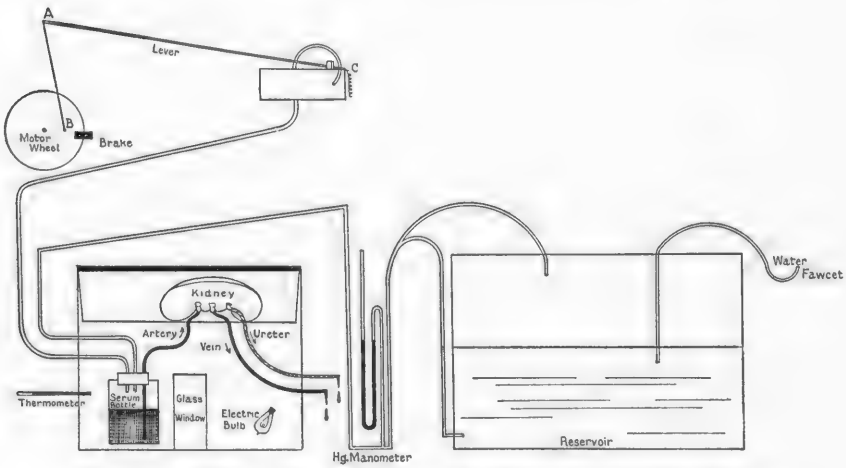
No. of section.	Time in moist chamber in min.	Weight before.	Time in fluid in hrs.	Time in moist chamber in min.	Weight after.	Difference in weight.	Per cent. of increase in weight.
1	10	1.225	1	10	1.342	0.117	
2	12	2.309	1	12	2.555	0.146	
3	15	1.622	1	15	1.800	0.178	
4	17	1.525	1	17	1.650	0.125	
5	19	2.020	1	19	2.235	0.215	
6	21	1.725	1	21	1.915	0.190	
Total weight = 10.426				Total increase = 0.971			9.3

From table III it will be seen that Adler's solution gave rise to too great an edema. Another fluid, therefore, was tried in which twice the amount of each of the solid ingredients recommended by Adler was used. This was tested in similar fashion to the other fluids and was considered suitable (table IV).

TABLE IV.

No. of section.	Time in moist chamber in min.	Weight before.	Time in fluid in hrs.	Time in moist chamber in min.	Weight after.	Difference in weight.	Per cent. of increase in weight.
1	14	1.057	1	14	1.057	0	
2	17	1.59	1	17	1.619	0.029	
3	20	1.662	1	20	1.645	−0.017	
4	22	0.982	1	22	0.987	0.005	
5	25	1.004	1	25	1.009	0.005	
6	28	0.752	1	28	0.745	−0.007	
Total weight = 6.997				Total increase = 0.015			0.21

Attention was therefore turned to devising an apparatus that would keep the organ and injecting fluid at body heat during the perfusion and supply the pressure and pulse necessary to circulation (text-figure 1).



Explanation of Text-Figure 1.—As the water from the tap ran into the reservoir, it generated pressure in the upper part of the reservoir, which was thence transferred to the manometer and serum bottle in the perfusion box.

As the wheel on the motor rotated, it moved the lever, AC. This let the air out intermittently from the serum bottle through the pulse tube. The speed of the pulse was regulated by the brake on the wheel. The size of the pulse was regulated by the length of the crank, AB. The pressure thus escaping was compensated for by the tap water which was allowed to run slowly into the reservoir.

The water pressure was necessary because compressed air from a power pump was not available.

At first it seemed possible to estimate the flow from the ureter simultaneously with the flow from the vein. Cannulæ were devised which fitted into the ureter, vein, and artery, and attempts were made to establish the rate of flow from the ureter and vein of normal kidneys with a pressure of 100 millimeters of mercury in diastole and 110 millimeters of mercury in systole, and a pulse of 120 a minute. Several normal kidneys were perfused. The fluid ran easily from the ureter and vein, but when paraffin sections were made of the kidneys, it was found that the cells had shrunken. This histological change seemed to be sufficiently great to eliminate the use of this modification of Adler's fluid, and search for a better perfusing fluid was consequently resumed. Brodie's suggestion of using defibrinated blood was now tried; but here again objections were met with. The blood did not flow rapidly enough through the kidney vessels. This, it was thought, might be due to clotting which in turn might be brought about by the liberation of fibrinoplastic ferment from the kidney during perfusion. Consequently a fresh salt solution extract of the kidney was made and added to the defibrinated blood. This gave rise to clotting. The blood was then filtered and used. Even then, however, the flow from the vein was very slow, while no flow came from the ureter.

It was then thought that diluting the defibrinated blood with salt solution, as recommended by Langendorff and his students, might give rise to a fluid that would flow more rapidly. Dilutions composed of one part of blood to two parts of salt solution, of one part of blood to three parts of salt solution, and one part of blood to four parts of salt solution, were tried, but all proved to be unsatisfactory for the same reasons that eliminated the use of defibrinated blood. It may be stated here that the change in the color of the blood from bright red to purplish red as it passed through the kidney was noticed. This has also been seen by Brodie and other investigators.

The work done so far led to the conclusion that Adler's fluid as described by him was the most suitable for the following reasons: (*a*) changes occurring would be alike in all kidneys; (*b*) the ingredients contained in the fluid were more thoroughly understood

than those contained in preparations in which blood was used; (*c*) the fluid could be easily obtained.

PERFUSION OF THE KIDNEY WITH ADLER'S SALINE SOLUTION.

The rabbit was etherized, the abdomen opened in the median line, the left kidney exposed, and the left renal artery and vein were tied off respectively at their attachments to the aorta and vena cava, with the animal still alive. The kidney was perfused with Adler's solution, warmed to 40° C. This was done to prevent an impediment to the flow of the solution because of post-mortem clotting. The animal was bled to death from the right femoral vessels, and the blood was collected aseptically, as it was thought that at some future date the serum might be used.

The spinal cord was then severed at the cervical vertebræ and at the pelvis, and that portion of the rabbit was taken out of the skin and laid on a tray. The kidney was protected and kept moist by a flap of the left abdominal wall. A cannula was inserted into the left ureter as quickly as possible and the tray with that portion of the rabbit was placed in the box. Previous to this the Adler's solution that was to be used had been filtered and warmed to 40° C. and placed in the box. Attachments were then made between the bottle containing Adler's solution, the cannula in the renal artery, the cannulæ in the renal vein and ureter, and two glass cannulæ which protruded from the side of the box. The box was closed, the pulse motor started, and regulated with the brake to 120 pulsations a minute. The tap was turned on until the pressure in the reservoir, as registered in the manometer, reached 100 millimeters of mercury, and the clamp was removed that kept the pressure from the bottle containing Adler's solution. A flow of water from the tap into the pressure tank was then so regulated that it compensated for the pressure which escaped from the solution bottle through the pulse tube, and the flow from the vein and ureter was estimated as the fluid escaped in drops from the vein and ureter cannulæ in the side of the box. The fluid came out in drops which were counted, as a rule, at intervals of five minutes. The temperature of the box was kept between 38° and 42° C. by means of the electric bulb. In making connections between the fluid and the renal artery, great care was taken to exclude all bubbles.

TABLE V.[1]

This shows the result of the perfusion of six normal kidneys by the method described above.

No. of rabbit.	13		23		28		30		31		32	
	B.	U.	B.	U.	B.	U.	B.	U.	B.	U.	B.	U.
After 5 min.	—	—	23	5	42	11	19	6	70	15	24	2
After 10 min.	24	6	24	2	47	15	24	9	70	14	24	2
After 7 min.	—	—	—	—	—	—	—	—	—	—	—	—
After 12 min.	—	—	—	—	—	—	P.24	9	—	—	—	—
After 15 min.	25	6	P.13	—	54	—	24	8	70	14	24	2
After 17 min.	P.12	2	P.11	½	—	—	—	—	P.57	9	P.18	4
After 20 min.	15	5	—	—	53	11	24	8	77	—	24	4
After 22 min.	18	4	10	—	—	—	—	—	P.49	6	—	—
After 25 min.	20	4	10	—	52	14	26	6	67	—	27	5
After 30 min.	P.15	2	10	—	50	13	29	7	50	5	32	4

In contradistinction to the above experiments in which the spinal column was severed in the region of the cervical vertebræ, the following kidneys are from normal rabbits the spinal columns of which were severed just below the diaphragm. This seemed to give rise to an increase in the flow and was considered to be due to the damage done to the kidney nerve supply.

TABLE VI.

No. of rabbit.	21		26		27	
	B.	U.		U.	B.	U.
After 5 min.	—	—	37	16	40	7
After 7 min.	P.64	7	—	—	40	7
After 10 min.	P.64	—	—	—	—	—
After 12 min.	66	—	P.37	11	P.38	7
After 15 min.	64	—	P.30	7	40	7
After 20 min.	58	—	22	4	P.39	7
After 25 min.	45	—	22	4	37	4

In the following series nephropathy was produced by cantharidin.

Rabbit 25 received subcutaneously 2 mgm. of cantharidin in acetic ether at 11 A. M. Owing to the condition of the rabbit, irrigation was started at 2.23 P. M.

[1] In the following tables B = drops from vein cannula in ½ min.; U = drops from ureter cannula in ½ min.; P = pulse of 120 a minute; — indicates that the drops were not estimated.

TABLE VII.

Rabbit 25.

	B.	U.
After 10 min.....	45	
After 12 min.....	67	No flow.
After 20 min.....	The pressure ran up to 144 mm. of mercury because of inattention. It was immediately brought down to 100 mm.	
After 22 min.....	110	

From here on the flow became so rapid that the drops could not be counted.

In the following series nephropathy was produced with potassium chromate.

Rabbits 24, 29, and 34 were given 5 mgm. of potassium chromate subcutaneously twenty-four hours before being perfused. Rabbit 33 had 5 mgm. of potassium chromate injected subcutaneously on each of two consecutive days and was perfused on the third.

TABLE VIII.

No. of rabbit.	24		29		33		34	
	B.	U.	B.	U.	B.	U.	B.	U.
After 5 min.....	—	—	57	15	120	24	51	16
After 10 min.....	—	—	62	13	130	26	51	23
After 12 min.....	31	4	—	—	—	—	P.61	23
After 15 min.....	32	5	60	11	70	14	68	21
After 17 min.....	35	8	—	—	—	—	—	—
After 20 min.....	57	8	57	8	35		64	16
After 22 min.....	P.57	6	—	—	—	—	—	—
After 25 min.....	—	—	57	7	14	2	59	14
After 27 min.....	55	4	—	—	—	—	—	—
After 30 min.....	—	—	48	7	13	1	61	7
After 35 min.....	60	2	—	—	—	—	55	5
After 37 min.....	P.59	2	—	—	—	—	—	—

The following series of rabbits was found, at autopsy, to have chronic nephropathy (table IX).

TABLE IX.

No. of rabbit.	17		18		19		20	
	B.	U.	B.	U.	B.	U.	B.	U.
After 5 min.....	34	5	71	44	—	—	—	—
After 10 min.....	P.38	4	—	—	63	7	38	—
After 15 min.....	49	—	37	46	P.65	7	38	—
After 20 min.....	—	—	P.35	24	64	8	P.39	—
After 25 min.....	—	—	P.40	7	50	3	—	—
After 30 min.....	—	—	—	—	P.49	3	40	—
After 35 min.....	—	—	—	—	46	3	38	—
After 40 min.....	—	—	—	—	—	—	P.36	—

From the tables it will be seen that the fluid used was unsatisfactory because the difference in flow between two rabbits of the same series, as is seen in table V, rabbits 13 and 31, and table VIII, rabbits 24 and 33, was very marked. This difference could not be accounted for by an error in technique or by macroscopical or microscopical change.

Furthermore, regardless of the changes in cells produced by the drug used for the production of nephropathy, the histological changes caused by perfusion with Adler's solution were so great that dependence could not be placed on the results obtained. The next fluid that was tried was rabbit blood serum. This was found to flow through rapidly and to give rise to very little change in the cells. Its vasoconstrictor (8) and vasodilator (9) action, being present only during the first few minutes of the perfusion, did not interfere with the final results. Serum, it was found, gave rise to no flow of fluid from the ureter, so the estimation of the ureter flow was given up.

The following precautions were taken with the serum: (1) it was collected aseptically and kept in an ice-chest when not in use; (2) it was filtered through eight layers of gauze before every perfusion; (3) the serum used for perfusion of the kidneys in which nephropathy had been produced by a drug was obtained from normal rabbits and from rabbits in which the nephropathy had been induced by the same drug; thus, the possibility of the drug having an untoward influence on the circulation of the serum was eliminated.

To test this point further, a normal kidney was perfused with cantharidin serum, the greatest impediment to the flow being caused by cantharidin nephropathy, and the flow in this case did not differ from that in which a normal serum and a normal kidney were used. Thus it was concluded that though nephrotoxic substances are present in the serum of animals with experimental nephritis (10), they have little immediate influence on the flow of serum through a normal kidney.

The method of perfusion was now somewhat altered. The rabbit was etherized and bled to death through the right femoral vessels. The abdomen was opened. The intestines, liver, and

stomach were removed. The spinal column was then severed below the diaphragm and above the pelvis. This section of the rabbit, with the kidneys *in situ,* was taken out, cannulæ were introduced into the left renal artery and vein, and the whole was put into the box in which the bottle containing serum had already been placed. Attachments were made between the source of pressure, a mercurial manometer, the bottle containing the serum, the cannulæ in the renal artery, the cannulæ in the renal vein, and a rubber tube the extremity of which fitted to another glass cannula which protruded from the side of the box at E (text-figure 1). The serum was then subjected to pressure.

The use of the same amount of pressure throughout the whole series of perfusions was found to be impracticable; serum sent through a normal kidney under eighty-eight millimeters of pressure flowed from the vein in a stream, while that sent through some of the diseased kidneys (table XIV) under the same pressure came out at the rate of ten drops a minute. Thus, were the same pressure to be used throughout, the only way that suggested itself by which the rapidity of the flow could be estimated and compared was to find how much time it took the flow from each kidney to fill a vessel of a known capacity. This method was thought inadvisable for the following reasons: (*a*) the greater time required for the flow from the diseased kidney to fill the vessel would have made it possible for changes, due to disintegration, to take place in the kidney; (*b*) an attempt made to shorten this time would have necessitated the use of a much greater pressure than normally (table XIII).

In all cases as little pressure as possible was used. The serum was warmed to 40° C. before being put in the box, and the temperature of the box was kept between 38° and 42° C. At an interval of five minutes the drops coming out of the cannulæ in the box were counted. The same cannulæ were used throughout. The cannula introduced into the artery was large enough to allow the serum to distend slightly the artery before entering the kidney. The cannula introduced into the vein was large enough to allow a free circulation, so that during the experiments the vein back of this cannula was never dilated with retained serum. It was found that the easiest way to introduce a cannula into the renal artery was by entering the renal artery from the aorta. Before introducing the

cannula, a fine pair of forceps was pushed in so as to dilate the entrance to the vessel. This was found necessary because in certain cases the introduction of the cannula into the artery tore off the endothelial lining of the renal artery from that of the aorta. This endothelial lining would then mass up in front of the cannula causing an obstruction to the stream.

No pulse was used in this series, as the previous experiments led to the conclusion that a pulsating pressure made no difference in the rate of flow sufficiently great to warrant its use. This differs from the conclusion arrived at by Sollmann. It seemed to me that the amount of flow within a given time through the kidney depended on the mean arterial pressure. If this exceeded the normal pressure, the flow naturally would be greater, otherwise it would be less. As heretofore, all bubbles were carefully avoided.

Landergren and Tigerstedt (11) have shown that under the influence of a diuretic the blood flowing through the kidney in one minute is equal to its weight.

As in a 1,500 gram rabbit each kidney weighs from five to seven grams, the flow of blood through this organ would average between seventy and ninety-eight drops a minute. Serum, as might be expected and as is shown by the following experiments, flows through much more rapidly.

The following tables show the results obtained from the present study.

TABLE X.

The following rabbits were normal.

The perfusion of rabbits 36 and 38 was carried on uneventfully.

In rabbit 37 the introduction of the cannula into the artery denuded part of the endothelium thus blocking the stream to a certain extent, consequently the cannula had to be reintroduced. The delay thus occasioned may have had some influence on the flow.

No. of rabbit.	36		37		38	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	66 mm.	70	54 mm.	21	44 mm.	Over 120
After 10 min.	66 mm.	Over 120	54 mm.	75	44 mm.	Over 120
After 15 min.	44 mm.	76	44 mm.	70	44 mm.	70
After 20 min.	44 mm.	63	44 mm.	70	—	—
After 25 min.	44 mm.	71	44 mm.	52	44 mm.	64
After 30 min.	44 mm.	65	44 mm.	55	—	—
After 35 min.	44 mm.	64	44 mm.	47	44 mm.	50
After 40 min.	—	—	44 mm.	36	—	—

TABLE XI.

The following rabbits had acute uranium nitrate nephropathy.

Rabbit 39 received subcutaneously 10 mgm. of uranium nitrate in solution on three consecutive days, and was killed on the fourth day. Rabbit 40 received subcutaneously 10 mgm. of uranium nitrate in solution on four consecutive days, and was killed on the fifth day. Rabbit 41 received subcutaneously 10 mgm. of uranium nitrate in solution on two consecutive days, and was killed on the third day.

No. of rabbit.	39		40		41	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	54 mm.	55	54 mm.	44	54 mm.	37
After 9 min.	54 mm.	89	54 mm.	70	54 mm.	90
After 10 min.	44 mm.	43	44 mm.	39	44 mm.	50
After 15 min.	44 mm.	36	44 mm.	24	44 mm.	19
After 20 min.	44 mm.	28	44 mm.	15	44 mm.	19
After 25 min.	44 mm.	18	44 mm.	13	—	—
After 30 min.	54 mm.	13	44 mm.	12	—	—

TABLE XII.

The following rabbits had acute potassium arsenate nephropathy.

Rabbits 50 and 51 received subcutaneously ten milligrams of potassium arsenate and were autopsied at the end of thirty minutes. Both rabbits developed albuminuria.

No. of rabbit.	50		51	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	88 mm.	17	88 mm.	16
After 10 min.	66 mm.	10	66 mm.	19
After 15 min.	66 mm.	13	66 mm.	21
After 20 min.	66 mm.	18	66 mm.	22
After 25 min.	——	——	66 mm.	28

TABLE XIII.

The following rabbits had acute potassium chromate nephropathy.

Rabbit 42 received subcutaneously 5 mgm. of potassium chromate solution on two consecutive days and was autopsied on the third day. A longer time than usual was allowed to elapse between the death of the rabbit and the perfusion of the kidney.

Rabbit 43 received subcutaneously 5 mgm. of potassium chromate solution on two consecutive days and was autopsied on the third day. Rabbits 44 and 45 were treated similarly.

No. of rabbit.	42		43	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	66 mm.	16	—	—
After 10 min.	66 mm.	12	—	—
After 15 min.	66 mm.	13	—	—
After 20 min.	66 mm.	13	154 mm.	4
After 25 min.	—	—	154 mm.	5

No. of rabbit.	44		45	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	—	—	88 mm.	20
After 10 min.	—	—	88 mm.	19
After 15 min.	120 mm.	1	88 mm.	30
After 20 min.	—	—	—	—
After 25 min.	120 mm.	15	—	—

TABLE XIV.

The following rabbits had acute cantharidin nephropathy.

Rabbit 47 received subcutaneously 2 mgm. of cantharidin dissolved in acetic ether. The rabbit was killed twenty-four hours later. Rabbit 48 received subcutaneously 1.5 mgm. of cantharidin and was autopsied twenty-four hours later.

No. of rabbit.	47		48	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	88 mm.	—	88 mm.	4
After 10 min.	88 mm.	6	88 mm.	6
After 15 min.	88 mm.	7	88 mm.	7
After 20 min.	88 mm.	11	88 mm.	8

TABLE XV.

The following rabbits had acute diphtheria nephropathy, both having received a unit of toxin per kilo of body weight two days before autopsy.

No. of rabbit.	22		0	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	80 mm.	4	80 mm.	20
After 10 min.	80 mm.	11	44 mm.	18
After 15 min.	80 mm.	24	44 mm.	25
After 20 min.	80 mm.	24	44 mm.	24
After 25 min.	80 mm.	25	—	—

Table XVI represents the average pressure and rate of flow used in tables X to XV, inclusive.

TABLE XVI.

	Normal.		Uranium nitrate.		Potassium arsenate.		Potassium chromate.		Cantharidin.		Diphtheria toxin.	
	Pressure.	Drops.	Pressure.	Drops.	Pressure.	Drops.	Pressure.	Drops.	Pressure.	Drops.	Pressure.	Drops.
After 5 min.	54 mm.	70	54 mm.	45	88 mm.	16	76 mm.	18	88 mm.	4	80 mm.	12
After 10 min.	54 mm.	105	54 mm.	80	66 mm.	14	76 mm.	15	88 mm.	6	62 mm.	14
After 15 min.	44 mm.	72	44 mm.	26	66 mm.	17	80 mm.	14	88 mm.	7	62 mm.	24
After 20 min.	44 mm.	77	44 mm.	20	66 mm.	19	110 mm.	8	88 mm.	9	62 mm.	24
After 25 min.	44 mm.	62	44 mm.	15	66 mm.	28	132 mm.	10	—	—	80 mm.	24
After 30 min.	44 mm.	56	44 mm.	12	—	—	—	—	—	—	—	—

HISTOLOGY.

In all cases sections of both perfused and non-perfused kidneys were run through paraffin and a microscopical examination was made. A complete description of the changes occurring in kidneys in which nephropathy had been produced by the various drugs used in the experiments has been given by many investigators. Only such changes, therefore, will be mentioned as were thought to have direct bearing on the problem.

As has already been stated, serum was used as it was found to produce the least change in the kidney cells; but the epithelium of the kidney is not indifferent even to such a fluid. The examination of the perfused and non-perfused kidneys from the same normal animal, subjected to precisely the same histological treatment, showed in the perfused kidney swelling and hydropic degeneration which is confined to the secondary convoluted tubules, the ascending arm of Henle, and the small collecting tubules. The epithelial cells in these tubules show larger and smaller, usually single, vacuoles. Other changes that arise by perfusion of the normal kidney are the removal of the blood, the slight accentuation of the separation between the tubules, and the bringing out with great clearness of the histological details of the empty glomeruli. These changes were produced by a perfusion which lasted no longer than thirty-five minutes; but even during this short time the changes produced seemed to offer some impediment to the flow.

Changes in the Kidneys in Which Nephropathy was Produced by Cantharidin.—In the perfused kidney the glomeruli showed a

diminution in the calibre of the blood vessels due to the swelling and proliferation of the lining endothelial cells, many nuclear figures being seen, and the presence in the capillaries of a large number of leucocytes, some of which showed fragmented nuclei. In the tubules there was a marked swelling of the epithelium which caused a narrowing of the intertubular spaces. The difference between the perfused and the non-perfused cantharidin kidneys was not marked. In the perfused kidney there seems possibly a somewhat greater degree of swelling of the degenerated epithelium, while the glomeruli in the non-perfused kidney contain more red cells and a greater number of leucocytes.

Changes in the Kidneys in Which Nephropathy was Produced by Uranium Nitrate.—The glomerular lesions in the uranium nitrate kidneys are in marked contrast with the cantharidin. The glomeruli in the perfused kidney are free from cells, the only change in contrast with the normal being a slight swelling of the capillary endothelium. The epithelial degeneration is more marked; there is extensive cell desquamation and necrosis extending over areas of considerable size. There is, however, a slight variation in the different cases, one case showing marked swelling and proliferation of both endothelium and covering epithelium.

Changes in the Kidneys in Which Nephropathy was Produced by Potassium Chromate.—The glomerular lesions in the potassium chromate kidney approach in intensity those of the cantharidin kidney. There is obstruction of capillaries due to cell accumulations within them and endothelial proliferation. The degenerative changes in the convoluted tubules are not so marked, there being but little actual necrosis. There is, however, considerable swelling.

Changes in the Kidneys in Which Nephropathy was Produced by Potassium Arsenate.—In the short time that elapsed between the administration of potassium arsenate and the perfusion of the kidneys, a swelling of the endothelium in the glomeruli had taken place.

Changes in the Kidney in Which Nephropathy was Produced by Diphtheria Toxin.—The lesions are essentially degenerative in character, affecting all the tubules, chiefly the convoluted tubules, the primary more strongly than the secondary. There is also a con-

siderable degree of interstitial edema, very marked in certain foci. All tubules are effected to about the same degree; the epithelium is swollen, granular, and vacuolated; the nuclei stain poorly and in places the epithelium is converted into granular masses entirely filling the tubules. The glomeruli are, for the most part, unchanged, though the vessels often contain more than the usual number of cells. Some are lymphoid, others are polynuclear in form.

It is evident in these cases that an impediment to the circulation may be produced in a number of pathological conditions in the kidney, the two ways most evident being by swelling of the epithelium producing pressure upon the kidney, and changes in the glomeruli acting by the diminution in capillary calibre, this being most obvious in the cantharidin kidney. The combination of the two conditions, as in the cantharidin kidney, will produce the most marked impediment.

CONCLUSIONS.

1. Blood serum is the most satisfactory perfusing fluid available.

2. There is some impediment to the circulation of serum through the vessels of kidneys in which nephropathy has been produced by uranium nitrate, potassium chromate, potassium arsenate, cantharidin, or diphtheria toxin.

3. The histological changes in the cells of these nephropathic kidneys—the swelling of the epithelium and the changes in the glomeruli—are such as would produce obstruction to the circulation.

4. The impediment to the circulation is greatest in those kidneys in which the above mentioned histological changes are most marked.

5. In the kidneys in which the drug used has caused a destruction of the cells, the impediment is less marked than in those where the cells are swollen but otherwise intact.

PART II.

THE OBSTRUCTION AT VARIOUS STAGES OF NEPHROPATHY.

Having found that nephropathy caused an impediment to perfusion with serum, the following series of experiments were conducted in order to determine the amount of impediment at various stages of the process.

SERIES I.

The following rabbits received subcutaneously 10 mgm. of potassium chromate per kilo of body weight and were autopsied as follows:

Rabbits 1 and 2, 24 hours after injection.
Rabbits 3 and 4, 48 hours after injection.
Rabbits 6 and 7, 72 hours after injection.
Rabbit 43, 120 hours after injection
Rabbit 29, 168 hours after injection.

No. of rabbit.	1		2		3		4	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 3 min.	80		80		80		80	
After 5 min.	60	5	50	18	60	8	60	8
After 10 min.	80	16	60	18	60	10	60	10
After 15 min.	80	9	60	17	60	9	60	11
After 20 min.	80	2	60	18	60	9	60	9
After 25 min.	100	3	60	16	60	5	60	9

No. of rabbit.	6		7		43		29	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 3 min.	80		70		60		60	
After 5 min.	40	34	40	21	60	42	60	15
After 10 min.	44	30	40	29	60	80	60	95
After 15 min.	40	28	40	28	40	37	40	46
After 20 min.			40	24	40	34	40	47
After 25 min.			40	22	40		40	45

The following table presents the average of the results obtained.

Hrs. after injection.	24		48		72		120		168	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 3 min.	80		80		75		60		60	
After 5 min.	55	11	60	8	75	27	60	42	60	15
After 10 min.	70	17	60	10	42	29	60	80	60	95
After 15 min.	70	13	60	10	40	28	40	37	40	46
After 20 min.	70	10	60	9	40	24	40	34	40	47
After 25 min.	80	9	60	7	40	22	—	—	40	45

SERIES II.

The following rabbits had a subcutaneous injection of 0.5 mgm. of cantharidin dissolved in acetic ether, per kilo of body weight, and were autopsied as follows:

Rabbits 25 and 98, 24 hours after injection.
Rabbits 15 and 47, 48 hours after injection.
Rabbits 81 and 52, 72 hours after injection.
Rabbits 48 and 97, 120 hours after injection.

No. of rabbit.	25		98		15		47	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum	Pressure.	Drops of serum.
After 5 min.	60	5	80	9	80	3	40	7
After 10 min.	80	17	80	11	80	5	40	7
After 15 min.	40	10	80	14	80	5	40	9
After 20 min.	40	9	40	9	80	6	40	10
After 25 min.	40	8	40	8	80	7	40	10

No. of rabbit.	81		52		48		97	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	70	5	60	30	60	88	40	90
After 10 min.	40	29	40	21	40	50	40	52
After 15 min.	40	31	40	31	40	40	40	52
After 20 min.	40	31	40	25	40	42	—	—
After 25 min.	40	31	40	24	—	—	—	—

The following table presents the average of the results obtained.

Hrs. after injection.	24		48		72		120	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min.	70	7	60	5	65	17	50	89
After 10 min.	80	14	60	6	40	25	40	51
After 15 min.	60	12	60	7	40	31	40	47
After 20 min.	40	9	60	8	40	28	40	42
After 25 min.	40	8	60	8	40	26	—	—

HISTOLOGY.

In this series of experiments potassium chromate was given in smaller doses and was found to have confined its actions more closely to the tubules. The changes produced have been described by Lyon (12) and Burmeister (13). The changes were most marked in the forty-eight and seventy-two hour kidneys, and from that time on they gradually subsided.

In the cantharidin kidneys the same lesions were noted that have already been spoken of in this paper. The changes were most prominent in the forty-eight hour kidneys, the other kidneys showing a gradual return to normal corresponding with the increased flow, as seen in the tables of series II.

In sections stained with methylene-blue and eosin, a great number of bacteria were found lodged in various parts of the kidney,

principally in the glomeruli. The organisms came from the serum which, in spite of all precautions, had become infected. It was at once thought that these organisms might have caused some impediment to the flow. A normal kidney was consequently perfused and the results obtained showed that the impediment, if any, was so slight as not to vitiate the general deduction. A normal rabbit was perfused with serum cloudy with bacteria. The results were as follows:

Normal rabbit, weight 1,450 gm.

	Pressure.	Drops of serum.
After 5 min.	55	36
After 10 min.	45	80
After 15 min.	50	100
After 20 min.	50	105

CONCLUSIONS.

1. In kidneys in which nephropathy has been induced by potassium chromate or cantharidin, the impediment offered to the flow of the perfusing serum is in direct ratio to the anatomical obstructive lesion, and tends to return to normal with cessation or healing of the process.

2. Bacteria, though present in great numbers, impede but little the flow of serum through the kidney.

PART III.

SPONTANEOUS NEPHROPATHY IN THE RABBIT.

Rabbits 8, 9, 17, 18, 19, 20, 28, 49, and 73 were found to have a nephropathy other than that induced. Rabbits 8, 9, 17, 18, and 28 had subcutaneous injections of potassium chromate, while rabbits 19, 20, 49, and 73 had no subcutaneous injection. Rabbits 17, 18, 19, and 20 were perfused with Adler's normal saline solution; serum was used with the others.

The results obtained from the perfusion of rabbits 17, 18, 19, and 20 are given in table IX, while the results obtained from the perfusion of kidneys 8, 9, 28, 49, and 73 are given in the following table.

HISTOLOGY (14).

The lesions are similar in all cases; a description will therefore be given only of cases 19 and 20 in which the injury was most

No. of rabbit.	8		9		28		49		73	
	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.	Pressure.	Drops of serum.
After 5 min..	60	10	60	13	80	15	80	38	60	27
After 10 min..	60	12	60	16	80	94	80	29	60	28
After 15 min..	60	7	60	8	40	34	80	25	60	28
After 20 min..	60	5	60	5	40	42	—	—	60	29
After 25 min..	—	—	—	—	—	—	—	—	60	29

marked. Case 19 shows a triangular area on the surface extending into the cortex. The base on the surface measures 1.5 mm. Within this area the glomeruli are intact. Bowman's capsules are somewhat dilated and there is a marked increase in the intertubular tissue, with atrophy of the tubules. Within many of the atrophic tubules are hyaline casts. Within this area there are foci of intense infiltration with lymphocytes. There is but little fibrillary increase in the interstitial tissue, and all the tubes show a decided narrowing of calibre and a low indifferent epithelium. The large areas are distinctly circumscribed. The cells in the area of cellular infiltration are well preserved, but here and there are a few small, scattered nuclear fragments. There are also pale bodies found without definite size or shape which possibly result from nuclear or cell fragmentation. Elsewhere in the kidney not connected with the surface and rather numerous in the middle of the cortex are similar areas of cell infiltration with tubular atrophy. The epithelium of the kidney is normal and there are no casts save in the atrophic tubules in the foci of disease.

In case 20 the lesion is much more pronounced, the various areas taking up a large part of the cortex; the lesions present a great deal of similarity to the interstitial foci in the human kidney, but with the important difference that there are no lesions in the glomeruli. The glomeruli are relatively more numerous in the affected areas, but there is no thickening of the capsule or vascular obliteration. The tubules seem to be preserved, and there are lumina in the very small canals. In one of the atrophic canals near the cortex there are found cells filled with dark green granules, all of the same size. In another tubule a number of desquamated epithelial cells

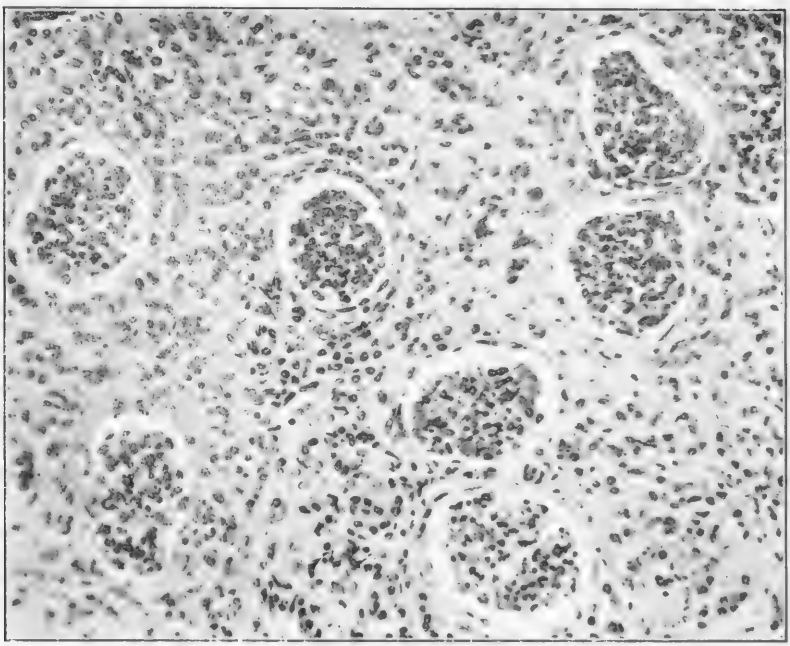


FIG. 1.

(Ghoreyeb : Mechanical Obstruction of the Kidney.)

are found which also contain this green granular pigment, and more or less of it is met with elsewhere.[2] In this case the largest lesion extends almost to the base of the papilla. There are occasionally dense hyaline masses found within the tubules at a distance from this area. The blood vessels are normal. The foci seem to represent areas where the atrophy of tubules is extensive but in which there is no actual destruction.

It is of interest to note that although all the rabbits showed histological lesions, none showed albumen or casts in the urine during life.

CONCLUSIONS.

1. Rabbits may have spontaneous nephropathy and show no casts or albumen in the urine.

2. If we leave out of consideration rabbits 17, 18, 19, and 20, which were perfused with Adler's solution, and rabbits 8, 9, 17, 18, and 28, which were injected subcutaneously with potassium chromate, rabbits 49 and 73 show that spontaneous nephropathy offers some impediment to the perfusing serum.

I am indebted to Dr. H. T. Karsner for suggestions and for the facilities of the experimental division of the laboratory of pathology.

BIBLIOGRAPHY.

1. Brodie, T. G., *Jour. Physiol.*, 1903, xxix, 266.
2. Sollman, T., *Am. Jour. Physiol.*, 1905, xiii, 241.
3. Loebell and Bidder, cited by Henle and Meissner, Berichte, 1863, 324; cited by Sollman, T., *loc. cit.*, p. 242.
4. Langendorff, O., and Lehmann, C., *Arch. f. d. ges. Physiol.*, 1906, cxii, 352.
5. Christian, H. A., *Boston Med. and Surg. Jour.*, 1908, clviii, 416.
6. Pearce, R. M., *Arch. Int. Med.*, 1910, v, 133.
7. Adler, H. M., *Jour. Am. Med. Assn.*, 1908, li, 752.
8. Brodie, T. G., *Jour. Physiol.*, 1900–1901, xxvi, 48.
9. Stewart, H. A., and Harvey, S. C., *Jour. Exper. Med.*, 1912, xvi, 103.
10. Pearce, R. M., *Jour. Med. Research*, 1908, xix, 269.
11. Landergren, E., and Tigerstedt, R., *Skand. Arch. f. Physiol.*, 1893, iv, 241.
12. Lyon, G., *Jour. Path. and Bacteriol.*, 1904, ix, 400.
13. Burmeister, Th., *Virchows Arch. f. path. Anat.*, 1894, cxxxvii, 405.
14. Ordway, T., Kellert, E., and Heusted, F. P., *Jour. Med. Research*, 1913, xxviii, 41.

EXPLANATION OF PLATE 1.

FIG. 1. Rabbit kidney showing spontaneous nephropathy.

[2] This pigment, the nature of which could not be determined, was found in one case only.

STUDIES UPON EXPERIMENTAL PNEUMONIA IN RABBITS.

V. The Rôle of the Leucocyte in Experimental Pneumonia. The Relation of the Number of Organisms Injected to the Mortality.*

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It has been definitely shown that lobar pneumonia may be produced in rabbits by the Meltzer method of intratracheal inoculation of cultures of pneumococci (1). The disease produced in this way runs a relatively rapid course, and, in the previous reports, it was noted that the animals died, as a rule, within a few days. It has been demonstrated further that in animals previously treated with benzol the pneumonia produced is much more rapidly fatal. Benzol, as is now well known, is a powerful leucotoxic substance not only destroying the leucocytes in the circulating blood but also affecting the bone marrow particularly, and the other hematopoietic organs to a less extent.

The rapidly fatal course of the pneumonia in animals in which the leucocytes had been previously destroyed to a great extent was further emphasized by a characteristic histological picture in the lungs of the animals. The pneumonic exudate consisted chiefly of fibrin and pneumococci, the latter in such large numbers that they densely stippled the entire section. These facts have naturally led us to associate the rapidly fatal outcome in these experiments with the diminished number of leucocytes. Before this association can be definitely established it is necessary to show that benzol aside from its action on the blood and hematopoietic organs does not lessen the resistance of the animals in some other way.

This question may be approached by the substitution of toluol

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for benzol.[1] Toluol differs from benzol only in having an additional methane radicle.

Benzol C_6H_6 Toluol $C_6H_5CH_3$

Toluol repeatedly injected into rabbits in exactly the same manner as benzol (one cubic centimeter of toluol + one cubic centimeter of olive oil per kilo of body weight, subcutaneously) is followed by certain local and general effects. Local sloughs may occur, the animals usually lose weight and in their general appearance are not unlike the animals treated with benzol. They may die from often repeated doses.

The two drugs, however, differ markedly in their effect upon the blood and blood-forming organs. No demonstrable change in the leucocyte count is present even after many doses of toluol, as may be seen from table I.

The changes in the bone marrow are of interest in this connection, for, as we shall show, the repeated injection of toluol is followed by a definite hyperplasia of this tissue. Several animals received six daily doses of toluol subcutaneously (one cubic centimeter in one cubic centimeter of olive oil per kilo of body weight) and were killed on the seventh day. The histological picture of the hematopoietic organs may be briefly stated as follows: The most marked changes appear in the bone marrow. Those in the lymph glands and other blood-forming organs are so slight that they may be omitted. The bone marrow shows a distinct hyperplasia involving particularly the myeloid elements. The cellular proliferation is so marked that the fatty marrow is almost entirely obliterated.

[1] The metabolism by the animal body of toluol differs from that of benzol. Toluol is oxidized to benzoic acid (C_6H_5COOH) and then combined with the glycocoll of the body is excreted as hippuric acid ($C_6H_5CO{\cdot}NHCH_2COOH$). Benzol is oxidized to phenol (C_6H_5OH) and to dioxybenzols (C_6H_4OHOH) which combine in the kidney with sulphuric and glycuronic acids to form phenolsulphuric ($C_6H_5OSO_3H$) and phenolglycuronic acids and the corresponding dioxybenzol compounds (Cushny, A. R., A Textbook of Pharmacology and Therapeutics, or the Action of Drugs in Health and Disease, 5th edition, Philadelphia, 1910, 448).

TABLE I.

Leucocyte Counts Before and After Injection of Toluol.

No. of animal.	Weight in gm.	Duration of experiment.	No. of injections of toluol.	White blood count before toluol.	White blood count after toluol.
3 A	1,200	8 days	6	8,200	8,400
3 B	1,570	8 days	6	8,800	9,700
2 A	1,750	8 days	6	9,000	9,900
2 B	1,870	8 days	6	10,500	14,000
46	1,130	8 days	5	7,600	13,000
59	1,720	7 days	4	7,000	5,700
60	1,580	7 days	3	7,800	5,800
61	1,850	7 days	6	16,900	13,400
62	1,360	7 days	5	8,900	6,900
63	1,290	7 days	6	15,700	13,200

The fact that following the repeated injections of toluol there is a definite hyperplasia of the bone marrow without an accompanying leucocytosis corroborates the impression gained in the work with benzol that the blood picture is not always an absolute index of the condition of the marrow.

With these preliminary tests in mind, several series of experiments, of which the following is an example, were performed.

Thirteen rabbits varying in weight from 1,300 to 1,900 gm. were used. Five were treated with repeated doses of benzol (three to six subcutaneous injections of 1 c.c. in an equal quantity of olive oil per kilo of body weight) until the circulating white blood cells were reduced, with but one exception, to between 420 and 720 per cubic millimeter. In one animal the cells still numbered 2,800 per cubic millimeter after six injections of benzol. Four animals were injected in a similar manner with toluol (three to six subcutaneous injections of 1 c.c. in an equal quantity of olive oil per kilo of body weight). The toluol and benzol groups of animals corresponded as nearly as possible in weight, nutrition, and general appearance.

Four control rabbits, corresponding likewise in weight and general appearance to four animals in each of the preceding groups, were included in the series. After the preliminary treatment with benzol and toluol all the animals including the controls were inoculated intratracheally with a culture of pneumococci.

The results of the experiment are presented in table II.

TABLE II.

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	56	18,000	Weight 1,550 gm.	60	7,800	Weight 1,580 gm.	66		Weight 1,390 gm.
Dec. 13			1.55 c.c. benzol subcutaneously			1.58 c.c. toluol subcutaneously			
Dec. 14			1.55 c.c. benzol subcutaneously			1.58 c.c. toluol subcutaneously			
Dec. 15			1.55 c.c. benzol subcutaneously			1.58 c.c. toluol subcutaneously			
Dec. 16		800							
Dec. 17		260							
Dec. 18		480							
Dec. 19		440	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,800	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,400	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20									
Dec. 21			Found dead 41 hrs. after injection. Lobar consolidation of left upper, $\frac{3}{4}$ of left lower lobe. Blood culture positive		11,000			10.000	
Dec. 22					14,000			13,400	
Feb. 15, 1913						Killed 58 days after injection			
March 7									Well 79 days after injection.

TABLE II.—*Continued.*

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	58	7,400	Weight 1,360 gm.	62	8,900	Weight 1,360 gm.	64		Weight 1,340 gm.
Dec. 13			1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 14			1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 15			1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 16		4,000	1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 17		1,300	1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 18		760							
Dec. 19		420	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		6,900	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		11,500	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20		380							
Dec. 21					16,100			14,900	
Dec. 22		860			17,900			12,900	
Dec. 23		260							
Dec. 24			Found dead 113½ hrs. after injection						
Jan. 24, 1913			Patchy consolidation of right lungs, most marked in the right lower. Beginning pleurisy. Blood culture positive						Killed 36 days after injection.
Mar. 7						Well 79 days after injection			

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	54	7,600	Weight 1,900 gm.	61	16,900	Weight 1,850 gm.	67		Weight 1,820 gm.
Dec. 13			1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 14			1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 15			1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 16		4,800	1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 17		4,200	1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 18		5,000	1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 19		2,800	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		13,400	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		6,600	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20					20,500			6,500	
Dec. 21		400			15,600			6,100	
Dec. 22		500						13,700	
Dec. 23		100	Died 92 hrs. after injection. Lobar consolidation $\frac{5}{6}$ of right lower and $\frac{1}{2}$ of right middle lobe. Fibrinous pleurisy. Blood culture positive						
Jan. 3, 1913						Died $14\frac{1}{2}$ days after injection. Areas of organized pneumonia and pleurisy in right lower lobe. Fibrinopurulent pericarditis. Blood culture positive			
Jan. 4									Died 16 days after injection. Extreme shaggy fibrinopurulent pleurisy and pericarditis, subdiaphragmatic abscess. Old lobar consolidation of right lower lobe. Blood culture negative.

TABLE II.—*Continued.*

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	55	15,800	Weight 1,560 gm.	59	7,000	Weight 1,720 gm.	65		Weight 1,550 gm.
Dec. 13			1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 14			1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 15			1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 16		1,900	1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 17		760							
Dec. 18		360							
Dec. 19		400	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,700	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,800	5 c.c. 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20					13,300				Died 28 hrs. after injection. Early lobar consolidation of right lung (3 lobes). Fibrinopurulent pleurisy and mediastinitis. Blood culture positive.
Dec. 21		260							
Dec. 22			Found dead 65 hrs. after injection. Lobar consolidation of right upper and middle lobes, central lobe, $\frac{3}{4}$ of right lower, $\frac{2}{3}$ of left upper with fibrinopurulent pleurisy. Blood culture positive		13,500				
Dec. 23									
Dec. 24						Found dead $113\frac{1}{2}$ hrs. after injection. Lobar consolidation of left upper, fibrinopurulent mediastinitis. Blood culture positive			

TABLE II.—*Continued.*

Date.	Benzol.		
	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	57	15,200	Weight 1,330 gm.
Dec. 13			1.33 c.c. benzol subcutaneously.
Dec. 14			1.33 c.c. benzol subcutaneously.
Dec. 15			1.33 c.c. benzol subcutaneously.
Dec. 16		5,500	1.33 c.c. benzol subcutaneously.
Dec. 17		3,200	1.33 c.c. benzol subcutaneously.
Dec. 18		1,240	1.33 c.c. benzol subcutaneously.
Dec. 19		720	
Dec. 20		380	
Dec. 21		360	
Dec. 22			
Dec. 23		560	
Dec. 24			Died 115 hrs. after injection. Lobar consolidation of central lobe with marked fibrinous pleurisy. Blood culture positive.

Analysis of Table II.—The first animal died twenty-eight hours after injection, and was a control. Occasionally during intratracheal inoculation definite injury is done to the larynx and trachea. Some of the animals die immediately of hemorrhage, others develop a septicemia which is fatal within twenty-four to thirty hours. It is possible that this may account for the rapid course of the infection in this animal. Following this five benzol rabbits died in rapid succession in 41, 65, 92, 113½, and 115 hours respectively after inoculation, averaging 85 hours. One toluol rabbit died 113½ hours after inoculation. The remaining three toluol and three control animals lived through the acute period of the disease, but one toluol rabbit died on the fifteenth day with a marked organizing fibrinopurulent pericarditis, and a control rabbit died on the sixteenth day with extreme shaggy organizing fibrinopurulent pleurisy and pericarditis. The similarity of the results in the toluol and control groups and their contrast to the results in the

benzol group are striking. Correspondingly the leucocyte counts in the control and toluol groups are in marked contrast to the counts in the benzol group. In the latter the counts were very low, while in the control and toluol animals the counts were either normal or increased.

These facts seem to indicate that if benzol and toluol are at all comparable, the lowered resistance of benzol animals to experimental pneumonia is dependent upon the action which benzol has upon the blood and blood-forming organs and not upon any other action it may exert.

Another important point concerning the relation of the leucocytes to the resistance of animals against pneumococcus infection is brought out by table II. It may be seen that all the animals receiving toluol responded to the inoculation of pneumococci with a leucocytosis. Not only was this leucocytosis present in all the toluol animals, but in those surviving the pneumonia the increase in the number of white cells was greatest, increasing from 5,800 to 14,000 and from 6,900 to 17,900. The results suggested the advisability of producing a leucocytosis in animals before the intratracheal inoculation of pneumococci to see whether or not the resistance of the animals to the infection is thereby increased. With the purpose of producing an artificial leucocytosis a large number of drugs were tried, but our experiments are still too unsatisfactory for a detailed report. However, it has been found that a leucocytosis can be produced in rabbits by repeated intravenous injection of nutrose (sodium caseinate), a protein salt compound first studied in this connection by Kier (2).

In a preliminary experiment the few animals treated with nutrose before inoculation with pneumococcus culture gave analogous results to those treated with toluol. While the experiments are still too meager to draw absolute deductions, the following series has warranted further investigation. Three control rabbits and two in which the leucocytes were increased from 10,700 to 26,700 and from 11,600 to 28,000, by repeated intravenous injections of nutrose were each inoculated intratracheally with five cubic centimeters of a twenty-five hour stock culture of pneumococcus

in pig serum broth containing approximately 375,000 organisms per cubic millimeter. The three controls died in 56 hours, 5 days, and 10 days, respectively. One nutrose animal died in 67 hours and the other is still living 47 days after injection.

There are still many questions to be answered relating to the rapidly fatal course of experimental pneumonia in animals rendered aplastic with benzol. Perhaps the most important one is whether or not the resistance of these rabbits may be increased by the intravenous injections of leucocyte suspensions. At any rate, these preliminary experiments in which it is shown that rabbits with low white blood counts are more susceptible than those with normal counts emphasize the importance of the rôle of the leucocyte in experimental pneumonia.

A third point of interest is also brought out by the table. It will be remembered that in previously reported studies upon experimental pneumonia in rabbits the disease ran a rapidly fatal course in the large percentage of cases and only in a very few was death delayed for a longer period of time. In this series, on the other hand, and throughout more recent experiments a considerable number of animals have survived the intratracheal inoculation of pneumococci. This has been of particular interest since the same culture has been used in all but a few instances, in which other strains were used to determine if these might also produce the characteristic picture. Experiments were conducted in the hope of determining the cause of this difference in mortality.

In one series twelve small rabbits were used. All were inoculated intratracheally with a stock culture of pneumococci in pig serum broth. In determining the number of bacteria each pair was counted as one organism. Of the three animals each receiving 5 c.c. containing approximately 33,000 organisms per cubic millimeter, two survived the infection. Of the three each receiving 5 c.c. containing approximately 50,000 per cubic millimeter, one survived the infection. The two animals each receiving 5 c.c. containing 100,000 organisms per cubic millimeter, the two each receiving 5 c.c. containing 130,000 per cubic millimeter, and the two each receiving 200,000 per cubic millimeter, all succumbed to the infection. In another series, as noted above, three animals receiving 5 c.c. with approximately 375,000 organisms per cubic millimeter all died, and in still another series all four animals receiving 5 c.c. with approximately 500,000 per cubic millimeter succumbed to the infection. On the other hand, of eight large animals weighing 1,710 to 2,780 gm., all recovered from the intratracheal inoculation of 5 c.c. of stock culture of pneumococci in pig serum broth containing 25,000

organisms per cubic millimeter in four instances, and 17,000 per cubic millimeter in the remaining four.

The experiments indicate definitely that the number of pneumococci injected have an important bearing upon the mortality in the inoculated rabbits, many or all of those receiving small doses recovering and many or all receiving larger doses succumbing.

From subsequent experiments we have been led to the belief that while the number of organisms injected is of great importance in determining the mortality it is not the only factor. Further work is being done to determine more definitely the other influences.

CONCLUSIONS.

1. The importance of the leucocyte in the resistance of animals to experimental pneumonia is emphasized by the fact that animals treated with benzol, a leucotoxic substance, rapidly succumb to the disease, while animals treated in like manner with toluol, a very similar chemical substance causing no leucopenia, show no decreased resistance.

2. The rôle of the leucocyte in the resistance of animals to experimental pneumonia is further emphasized by the fact that animals that respond to the pneumococcus infection with a leucocytosis, as occurs after the repeated injection of toluol, are more resistant to the pneumonia. Further, the hyperleucocytosis produced by repeated injection of nutrose before the production of pneumonia likewise seems to increase the resistance of the animals.

3. Experimental pneumonia is not necessarily fatal in rabbits. The factors determining the outcome of the disease are numerous; among these is the number of bacteria inoculated. Animals receiving small doses usually survive, while those receiving comparatively large numbers usually succumb.

BIBLIOGRAPHY.

1. Winternitz, M. C., and Hirschfelder, A. D., *Jour. Exper. Med.*, 1913, xvii, 657.
Hirschfelder, A. D., and Winternitz, M. C., *ibid.*, 666.
2. Kier, *Nord. Med. Ark.*, 1905, xxxviii, 1.

STUDIES UPON EXPERIMENTAL PNEUMONIA IN RABBITS.

VI. Studies in Immunity.*

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It has been shown that experimental pneumonia is not necessarily fatal in rabbits and that the number of pneumococci injected intratracheally is of importance in the outcome of the disease (1). If a small number of organisms are injected the animals usually recover, and if much large doses are employed they usually die. These facts were utilized to determine whether animals could be given repeated increasing doses of pneumococci intratracheally and whether an immunity could be thereby established.

The presence of protective substances in human serum during lobar pneumonia has recently been shown by Dochez (2), who reports the protection of white mice against many times the fatal dose of the homologous organism. He succeeded in doing this by the simultaneous injection of serum from patients with lobar pneumonia drawn at the time of and after the crisis. These protective substances were found to be present in the serum as long after the crisis as the patients were under observation (five to twenty days).

ACTIVE IMMUNITY.

In the experiments to be reported the rabbits were usually inoculated at intervals of two weeks, although in some cases a period of a month elapsed between injections. Five cubic centimeters of a twenty to twenty-eight hour culture of stock pneumococcus in pig serum broth was the quantity always inoculated. As an initial dose some of the animals received 17,000, others 25,000, others 100,000, and still others 150,000 organisms per cubic millimeter of

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culture fluid. The number of bacteria in subsequent doses was increased gradually to 500,000 per cubic millimeter. Inasmuch as the organisms grow out in pig serum broth to the number of 200,000 to 300,000 per cubic millimeter at best, the culture fluid was concentrated for the larger doses by centrifugalization.

A large number of animals were used in these experiments. The evidence of the active immunization may be best illustrated by the type experiment in table I.

It is seen that an animal recovering from one attack of pneumonia, the result of the intratracheal inoculation with pneumococci, may then withstand repeated doses. Further, if an animal that has recovered from an inoculation of a moderate number of pneumococci is subsequently repeatedly injected with carefully graded increasing doses, such a large number of organisms may then be tolerated as invariably proves fatal in control animals. This offers definite evidence of an immunity after experimental pneumonia.

Twenty-six rabbits were repeatedly inoculated intratracheally with pneumococci. The results are briefly presented in table II.

Analysis of Table II.—Apparently animals do not all react similarly to repeated intratracheal inoculations with pneumococci. The vast majority surviving the first injection will subsequently survive repeated ones. The number of injections that they tolerate is variable:

3 animals succumbed after 2 injections;
5 animals succumbed after 3 injections;
7 animals succumbed after 4 injections;
1 animal succumbed after 5 injections.

Of the animals killed or still alive:

1 animal survived 8 injections;
4 animals survived 6 injections;
2 animals survived 5 injections;
1 animal survived 4 injections;
2 animals survived 3 injections.

From the table it does not appear that the immunity established by repeated intratracheal inoculations of pneumococci is absolute, but, as we shall show, there are several facts which must be taken into consideration in this relation before a definite conclusion is reached.

TABLE I.

Active Immunization.

Date.	No. of animal.	Weight in gm.	No. of organisms injected intratracheally (5 c.c. culture).	Remarks.	No. of control animal.	Weight in gm.	No. of organisms injected intratracheally (5 c.c. culture).	Remarks.
Dec. 19, 1912	62	1,360	100,000 per c.mm.	Recovered				
Jan. 19, 1913			100,000 per c.mm.	Recovered				
Feb. 4			300,000 per c.mm.	Recovered	K	1,680	300,000 per c.mm.	Died 88 hrs. after injection; lobar consolidation of $\frac{1}{2}$ of right middle with beginning involvement of right lower lobe. Blood culture positive, post mortem.
Feb. 14			300,000 per c.mm.	Recovered	116	1,275	300,000 per c.mm.	Died 26$\frac{1}{2}$ hrs. after injection; early lobar consolidation of right lower and middle lobes with early fibrinous pleurisy. Blood culture positive.
Mar. 3			500,000 per c.mm.	Recovered	118	1,750	500,000 per c.mm.	Found dead 20 hrs. after injection. Lobar type of consolidation of upper portion of right lower with beginning involvement of right middle lobe; marked early fibrinous pleurisy. Blood culture positive.

TABLE II.

Animals Repeatedly Injected Intratracheally with Pneumococci.

No. of animal.	Weight in gm.	No. of inoculations.	No. of organisms per c.mm. (first and last injections).	Result.				Lungs.	Other lesions.	Post-mortem blood culture.
				Died.		Recovered.				
				Within 30 hrs.	After 30 hrs.	Killed.	Alive.			
6B		4	100,000 to 300,000	20 hrs.				Patch of old consolidation in left lower lobe. Early consolidation of right lower with beginning involvement of right upper and middle lobes	Early pleurisy with moderate effusion (right side)	Positive; few colonies.
20		3	100,000 to 150,000	28½ hrs.				Early consolidation of left upper and lower lobes	Chronic adhesive pleuritis (right side)	No growth.
22		4	100,000 to 300,000	Immediately				Abscesses in right middle and lower lobes	Extensive bilateral adhesive pleuritis; organizing pericarditis. Pneumothorax (right side)	No growth.
26		4	33,000 to 300,000	22 hrs.				Patch of old consolidation in left lower lobe. Early lobar consolidation of right lower and most of right middle lobe	Mild localized, organized pericarditis	No growth.
28		2	33,000 to 150,000	6½ hrs.				Lobar consolidation of left upper and lower lobes with patches in right lungs	Fibrinopurulent pleurisy, bilateral; fibrinopurulent mediastinitis; fibrinopurulent pericarditis	Positive.
31		3	50,000 to 150,000	15½ hrs.				Patch of old consolidation in right lower lobe. Engorgement and beginning consolidation of right upper, middle, and lower lobes	Organizing pleurisy over old consolidation	No growth.

No. of animal.	Weight in gm.	No. of inoculations.	No. of organisms per c.mm. (first and last injections).	Result.				Lungs.	Other lesions.	Post-mortem blood culture.
				Died.		Recovered.				
				Within 30 hrs.	After 30 hrs.	Killed.	Alive.			
51		4	100,000 to 300,000	18½ hrs.				Abscesses in left lower lobe and early lobar consolidation of right lower and portions of right middle and upper lobes	Pyopneumothorax (left side)	No growth.
72	2,250	4	17,000 to 300,000	16½ hrs.				Old consolidation of portions of right lower and middle lobes. Right lower congested	Bilateral adhesive pleuritis, adhesive pericarditis, organizing mediastinitis	No growth.
75	2,550	4	17,000 to 300,000	Immediately				Areas of old consolidation of both lower lobes. Emphysema of all lobes	Purulent and beginning fibrinopurulent pericarditis, adhesive pleuritis (left)	No growth.
D	2,060	5	100,000 to 500,000		75 hrs.			Areas of old consolidation in both lower lobes; areas of atelectasis	Chronic bilateral adhesive pleuritis	Positive; few colonies.
64	1,340	3	100,000 to 300,000		114 hrs.			Areas of old consolidation in right lower lobe; areas of atelectasis	Old organizing pleurisy over old consolidation	No growth.
71	2,780	4	25,000 to 300,000		7 dys.			Lobar consolidation (white) of ½ of central lobe	Chronic adhesive pericarditis; cardiac dilatation and hypertrophy. Organizing fibrinopurulent pleurisy over consolidated area	No growth.
74	1,710	3	17,000 to 300,000		8 dys.			Areas of old consolidation in both lower lobes	Organized fibrinopurulent pleurisy over old consolidated area. Marked distention of bladder adherent to gut showing stricture	No growth.

TABLE II.—*Continued.*

No. of animal.	Weight in gm.	No. of inoculations.	No. of organisms per c.mm. (first and last injections).	Result.				Lungs.	Other lesions.	Post-mortem blood culture.
				Died.		Recovered.				
				Within 30 hrs.	After 30 hrs.	Killed.	Alive.			
23		2	100,000 to ?		8 dys.			Lobar consolidation (gray) in right lower and middle lobes with older areas of consolidation in right lower	Shaggy fibrinopurulent pleurisy (bilateral) and mediastinitis	Positive; few colonies.
70	2,240	3	25,000 to 300,000		11 dys.			Areas of old consolidation in right lower lobes	Organized fibrinopurulent pleurisy over old consolidated area	No growth.
25		2	150,000 to ?		18 dys.			Abscess in right lower and central lobes with extension through diaphragm; old consolidation in $\frac{1}{2}$ of right middle and lower $\frac{3}{4}$ of right upper lobes	Marked fibrinopurulent pleurisy; subdiaphragmatic abscess	No growth.
2B	1,870	6	100,000 to 300,000			11 dys.		Abscess in $\frac{1}{3}$ of right lower lobe	Adhesive pleuritis (right side)	
60	1,580	3	100,000 to 300,000			11 dys.		Scattered areas of congestion and atelectasis		
52		5	100,000 to 300,000			11 dys.		Apparently normal		
73	2,380	4	17,000 to 300,000			11 dys.		Apparently normal		

TABLE II.—*Continued.*

No. of animal.[1]	Weight in gm.	No. of inoculations intratracheally.	No. of bacteria per c.mm. in 5 c.c. culture (first and last injections).	Result.			
				Died.		Recovered.	
				Within 30 hrs.	After 30 hrs.	Killed.	Alive.
21		8	100,000 to 500,000				10 days after last injection.
62	1,360	5	100,000 to 500,000				10 days after last injection.
66	1,390	6	100,000 to 500,000				10 days after last injection.
68	2,170	6	250,000 to 500,000				10 days after last injection.
69	2,540	6	250,000 to 500,000				10 days after last injection.
G	2,380	3	300,000 to 500,000				10 days after last injection.

The changes which take place in the thorax in animals dying after repeated inoculations will be discussed later, but here it may be said that fibrous pleurisy, pericarditis, mediastinitis, and organization within the lungs frequently occur.

In a number of animals (six) repeatedly inoculated, death followed injection so rapidly that its probable explanation appeared to be the mechanical injury to a cardiorespiratory system already handicapped by chronic lesions. Death in these animals was infrequently associated with a septicemia, and when a septicemia was present it was mild.

This fact deserves special emphasis, for, as we shall show, rabbits dying from a primary attack of experimental pneumonia invariably have a marked septicemia. For example, fifty-two rabbits dying within a few days after a primary attack all showed in smears and blood cultures made at autopsy the presence of the pneumococcus in large numbers. Furthermore, in the five cases in which blood cultures and smears were made repeatedly during the course of the disease the septicemia was found to increase in severity as death approached. This is illustrated in table III.

[1] At the same time that the animals were inoculated with the larger doses, control rabbits were similarly inoculated, and with one exception died within a few days.

TABLE III.

Extent of Septicemia.

Date.	No. of animal.	Weight in gm.	Pneumococcus injected intratracheally (5 c.c. culture).	Blood culture.	No. of organisms per c.mm. in animal's blood.	Remarks.
Feb. 19, 1913	116	1,275	300,000 per c.mm. Stock culture (S)			
Feb. 20, A.M.				+	Too few to count	Animal died 26½ hrs. after injection.
Feb. 20, P.M.				+	5.40 P.M. 9,000,000 per c.mm.	Blood smear made 10 to 15 min. after death of animal.
Jan. 19	92	1,290	20,000 per c.mm. (C)			Smear made immediately after death of animal (73 hrs. after injection).
Jan. 22				+	10,000,000 per c.mm.	
Jan. 19	77	1,680	375,000 per c.mm. (S)			
Jan. 20				+	Too few to count	
Jan. 22					Too few to count	
Jan. 24, 11.30 A.M.				+	350,000 per c.mm.	Smear made ½ hr. after death of animal, 5 days after injection.
Jan. 24, 6.30 P.M.				+	2,500,000 per c.mm.	
Jan. 19	76	1,730	375,000 per c.mm. (S)			
Jan. 20				+	Too few to count	
Jan. 21				+	2,000,000	Smear made immediately after death of animal (56 hrs. after injection).
Feb. 3	W		40,000 per c.mm. (C)			
Feb. 4				+	4,600,000	Smear made immediately following death of animal (32 hrs. after inoculation).

The increase in the number of organisms occurring in the blood in the fatal cases of primary pneumonia is striking. Animal 116, for example, received intratracheally five cubic centimeters of a culture of pneumococcus containing 300,000 organisms per cubic millimeter. In eight hours a septicemia had developed. At the end of eighteen hours the organisms in the blood were still too few to count accurately, but in the next nine hours they had increased to 9,000,000 per cubic millimeter.

As has been said, a septicemia is always present in the fatal cases of primary pneumonia. On the other hand, in a few cases subsequently recovering, a small number of organisms were present in the blood for a time, disappearing with the clinical recovery of the animal (table IV).

TABLE IV.

Animals Recovering from Septicemia.

Date.	No. of animal.	Weight. in gm.	5 c.c. pneumococcus intratracheally.	Blood culture.	No. of organisms per c.mm.	Result.
Jan 19, 1913	G	2,380	375,000 per c.mm.			
Jan. 20				Positive; few colonies	Too few to count	
Jan. 22					Too few to count	
Jan. 24				Positive; few colonies	Too few to count	
Jan. 28				No growth		
Mar. 14						Well 14 days after injection.
Feb. 19	117	1,640	300,000 per c.mm.			
Feb. 20				Positive; few colonies	Too few to count	
Feb. 21				Positive; few colonies	Too few to count	
Feb. 22				Positive; few colonies	Too few to count	
Feb. 23				No growth		
Feb. 24				No growth		Killed.

The lesions occurring in the animals that died or were killed after repeated injections deserve comment. Animals 60, 52, and 73, killed eleven days after three, four, and five injections, respectively, showed apparently normal lungs. Inasmuch as the animals manifested definite signs of respiratory distress, pain on pressure of the thorax, a tendency to fix the thorax on the affected side, etc., for a few days following each injection, it may be concluded that in rabbits complete resolution may take place after lobar pneumonia.

On the other hand, it will be seen that most of the animals showed the result of previous inoculations in the form of patches of organizing consolidation or abscesses in the lungs, chronic adhesive pleuritis, or empyema, purulent or organizing pericarditis, or chronic mediastinitis. The animals dying within six and a half to twenty-eight and a half hours after the last injection showed in addition early lobar consolidation. The sudden death in a few animals (Nos. 22, 75, and 72) in whom no septicemia or acute lesion in the lung was demonstrable may have been caused by the injection of a large amount of material into a lung already handicapped by a chronic process. This has been discussed above.

PASSIVE IMMUNITY.

Having demonstrated the fact that animals that have been repeatedly inoculated with pneumococci intratracheally possess an active immunity, an attempt was made to use their serum to immunize rabbits passively. Therefore a number of animals recovering from repeated doses of a stock pneumococcus culture inoculated intratracheally were bled and the serum was obtained aseptically. This serum was then inactivated by heating to 56° C. for half an hour, and refrigerated until used. In the experiments to be reported below some of the animals received the inactivated immune serum, while others received a mixture of two parts of inactivated immune serum, seven parts of 5 per cent. boric acid solution, and one part of 1 per cent. sodium oleate solution, a mixture employed and found efficacious by Lamar (3) in the prophylactic and specific treatment of pneumococcus meningitis in the monkey.

Two series of animals were inoculated with the immune serum

TABLE V.

Immune serum.				Therapeutic mixture.			Untreated.		
Date.	No. of animal.	Weight in gm.	Remarks.	No. of animal.	Weight in gm.	Remarks.	No. of animal.	Weight in gm.	Remarks.
Mar. 3, 1913	123	1,370	2 c.c. of inactivated immune serum intravenously; 5 min. later 5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally (homologous organism)	129	1,370	10 c.c. of therapeutic mixture intravenously; 5 min. later 5 c.c. of 28 hr. culture (homologous) of pneumococcus, 500,000 per c.mm., intratracheally	119	1,400	5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally. Blood culture no growth, 6 hrs. later.
Mar. 4			Blood culture no growth			Blood culture no growth			Blood culture positive, A.M. Blood culture positive, P.M.
Mar. 5			Blood culture no growth			Blood culture no growth			Blood culture positive.
Mar. 6			Blood culture no growth			Blood culture no growth			Blood culture positive.
Mar. 7			Blood culture no growth			Blood culture no growth			Died 91 hrs. after injection. Lobar consolidation (gray) of ½ of right middle and ½ of central lobe with involvement of upper portion of right lower. Purulent and fibrinopurulent bilateral pleurisy. Fibrinopurulent pericarditis and mediastinitis. Blood culture positive, post mortem.
Mar. 8			Blood culture no growth			Blood culture no growth			
Mar. 9			Blood culture no growth			Blood culture no growth			
Apr. 9			Well 30 days after injection			Well 37 days after injection			

TABLE V.—*Continued.*

Immune serum.				Therapeutic mixture.			Untreated.		
Date.	No. of animal.	Weight in gm.	Remarks.	No. of animal.	Weight in gm.	Remarks.	No. of animal.	Weight in gm.	Remarks.
Mar. 3, 1913	122	1,650	2 c.c. of inactivated immune serum intravenously; 5 min. later 5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally (homologous organism)	126	2,450	10 c.c. of therapeutic mixture intravenously; 5 min. later 5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally (homologous organism)	120	2,360	5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally. Blood culture no growth, 6 hrs. later.
Mar. 4			Blood culture no growth			Blood culture no growth			Blood culture no growth, A.M. Positive, P.M.
Mar. 5			Blood culture no growth			Blood culture no growth			Blood culture positive.
Mar. 6			Blood culture no growth			Blood culture no growth			Blood culture positive.
Mar. 7			Blood culture no growth			Blood culture no growth			Blood culture no growth.
Mar. 8			Blood culture no growth			Blood culture no growth			Blood culture positive.
Mar. 9			Blood culture no growth			Blood culture no growth			Died 140 hrs. after injection. Lobar consolidation (gray) of $\frac{1}{2}$ of right middle with involvement of upper portion of right lower lobe. Fibrinopurulent mediastinitis and peritonitis. Blood culture positive, post mortem.
Apr. 9			Well 37 days after injection			Well 37 days after injection			

TABLE V.—*Continued.*

Immune serum.				Untreated.		
Date.	No. of animal.	Weight in gm.	Remarks.	No. of animal.	Weight in gm.	Remarks.
Mar. 3, 1913	124	1,650	4 c.c. of inactivated immune serum intravenously; 5 min. later 5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally (homologous organism)	118	1,750	5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally. Blood culture no growth, 6 hrs. later.
Mar. 4			Blood culture no growth			Blood culture positive, A.M. Died 20 hrs. after injection. Lobar type of consolidation of ¼ of right lower with beginning involvement of other portions of lobe. Marked fibrinous pleurisy overlying. Blood culture positive, post mortem.
Mar. 5			Blood culture no growth			
Mar. 6			Blood culture no growth			
Mar. 7			Blood culture no growth			
Mar. 8			Blood culture no growth			
Mar. 9			Blood culture no growth			
April 9			Well 37 days after injection			
Mar. 3, 1913	125	1,380	4 c.c. of inactivated immune serum intravenously; 5 min. later 5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally (homologous organism)	121	1,300	5 c.c. of 28 hr. culture of pneumococcus, 500,000 per c.mm., intratracheally. Blood culture positive, 6 hrs. later.
Mar. 4			Blood culture no growth, A.M. Blood culture positive, P.M. Died 31 hrs. after injection. Lobar consolidation (red hepatization) of right lower lobe, fibrinopurulent pleurisy. Blood culture positive, post mortem.			Found dead 16 hrs. after injection. Lobar type of consolidation (early) in upper portion of right lower lobe and portion of right middle. Blood culture positive, post mortem.

and the homologous pneumococcus. The results were so similar that only one series will be reported (table V).

The four control animals died in 91, 140, 20, and 16 hours, respectively, averaging 67 hours. Only one of the four animals treated with immune serum died (31 hours after injection). Both animals treated with the mixture of immune serum, boric acid, and sodium oleate survived. None of the surviving animals gave a positive blood culture at any time after inoculation. This demonstrates conclusively the protective power of the immune serum.

CONCLUSIONS.

1. Rabbits recovering from one attack of experimental pneumonia possess an active immunity. Such animals may subsequently withstand repeated increasing doses of pneumococci intratracheally.

2. Death may supervene after any one of subsequent injections, but it seems to depend partly upon the chronic changes in the cardio-respiratory apparatus. It may at least be said that it is usually unassociated with a septicemia which is an invariable accompaniment of fatal primary lobar pneumonia.

3. The serum from animals actively immunized by the repeated intratracheal inoculations with pneumococci may be used successfully to confer a passive immunity against the homologous organism.

BIBLIOGRAPHY.

1. Kline, B. S., and Winternitz, M. C., *Jour. Exper. Med.*, 1913, xviii, 50.
2. Dochez, A. R., *Jour. Exper. Med.*, 1912, xvi, 665.
3. Lamar, R. V., *Jour. Exper. Med.*, 1912, xvi, 581.

A STUDY OF THE ADDITION OF CHOLESTERIN TO THE ALCOHOLIC EXTRACTS OF TISSUES USED FOR ANTIGENS IN THE WASSERMANN REACTION.*

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The non-specific character of the so called antigen used in the Wassermann reaction has permitted a wide variation in the selection of material from which it has been prepared. Consequently, the reaction has yielded varying results in the hands of different workers. It is the object of this communication to compare the results obtained with several of the more extensively used antigens.

In spite of much experimentation no constant antigen has been adopted by a large group of workers. Practically all have agreed that the alcoholic extracts of livers of syphilitic fetuses are the best. The same method of preparation from different fetal livers will, however, yield reagents of widely different qualities. Many of them are hemolytic, others contain an excess of non-specific anticomplementary substances, and others have only feeble fixing power, so that probably not half the preparations made from these tissues can be used. Frequently very good reagents can be prepared by simple alcohol extraction of the livers of non-syphilitic fetuses or stillborn babies. The irregularity in the strength of antigens prepared from livers, as well as the difficulty in obtaining fetal livers, has led many to use alcoholic extracts of hearts. For this purpose, human, beef, and guinea pig hearts have been mostly employed. Lesser (1) has used saline emulsions of the residue obtained by extracting dried human hearts with ether, and this has been found by Dembowski (2) to yield a better antigen than alcoholic extraction. Such an ether extract is now on the market.

In searching for the active substance, Noguchi (3) concluded that a pure lipoid which consisted of the ether-soluble, acetone-insoluble portion of an alcoholic extract of either hearts or livers was the most reliable antigen. His method of preparation yielded, however, substances of widely different properties, so that each extract had to be thoroughly tested before it was adopted as a standard reagent. Browning and McKenzie (4) recommended a slightly different technique for the preparation of the pure lecithin. They found that a lecithin prepared from beef liver had good antigenic properties, though some-

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what less active than the crude alcoholic extract from the same organ. The addition of cholesterin to the lecithin produced an antigen which was superior to either the lecithin or the crude alcoholic extract. These authors claimed that if a given serum absorbed more complement in the presence of a lecithin-cholesterin mixture than in the presence of lecithin alone, the serum was surely from a syphilitic subject. Cholesterin alone had practically no antigenic action. Sachs (5) applied the method of fortifying with cholesterin to crude alcoholic extracts of normal hearts. He extracted beef hearts with five volumes of alcohol, and to 5 c.c. of this extract he added 0.3, 0.4, or 0.5 c.c. of a 1 per cent. alcoholic solution of cholesterin. The optimum amount of cholesterin was determined by comparing the different mixtures with a standard antigen. Often alcohol was added to the mixture, for Sachs, as well as Browning and McKenzie, has claimed that the addition of a certain amount of alcohol will increase the activity of an antigen. Sachs states that this activity is due not only to the concentration of the lipoids, but to their relative proportions. He reports that the cholesterin-fortified extract of beef heart was as strong an antigen as the best alcoholic extract of syphilitic liver.

McIntosh and Fildes (6) found that an antigen composed of five parts of alcoholic extract of human heart plus four parts of 1 per cent. alcoholic solution of cholesterin was much superior to either an alcoholic extract of normal heart, an alcoholic extract of syphilitic liver, or the lecithin-cholesterin mixture of Browning. More important still, they determined that antigens prepared in this manner from twenty-four different human hearts had all practically the same antigenic and anticomplementary properties. Their results seem to indicate that the standard antigen should be a mixture of heart extract and cholesterin.

Since the optimum amount of cholesterin seems never to have been determined, it seemed desirable to ascertain it, and, in addition, to compare the cholesterin-fortified extracts from hearts and livers of different animals. The present communication is a report upon this aspect of the subject.

METHODS.

A slightly modified Wassermann technique was used. All quantities of the various reagents were one half the amounts originally described by Wassermann, Neisser, and Bruck (7).

Hemolytic System.—0.5 of a cubic centimeter of a 5 per cent. suspension of sheep cells was used as a standard. The rabbit serum anti-sheep amboceptor was standardized with a complement of known strength. By using diminishing quantities of the amboceptor with one previously determined unit of complement, the minimum hemolytic dose of the amboceptor was determined. The amboceptor was then diluted, so that 0.25 of a cubic centimeter contained one unit of amboceptor. It was found to be important to determine

this amount accurately, because comparatively slight variations in the amount of amboceptor used cause variation in the amount of complement necessary. Protocol I shows this point clearly.

PROTOCOL I.

Method of Determination of the Hemolytic Unit of Amboceptor.

Tube.	Amount of amboceptor dilution.	Complement, guinea pig serum 10 per cent.	Sheep cells 5 per cent.	All made up to 2.5 c.c. Incubation 1 hr. at 37° C.	Dilution of amboceptor.			
					1:250	1:300	1:350	1:400
1	0.5 c.c.	0.5 c.c.	0.5 c.c.		C.H.[1]	C.H.	C.H.	C.H.
2	0.4 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
3	0.3 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
4	0.25 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
5	0.2 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
6	0.1 c.c.	0.5 c.c.	0.5 c.c.		C.H.	N.C.H.	N.C.H.	Mod. H.
7	0.25 c.c.	0.4 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
8	0.25 c.c.	0.3 c.c.	0.5 c.c.		N.C.H.	C.H.	C.H.	C.H.
9	0.25 c.c.	0.25 c.c.	0.5 c.c.		Mod. H.	N.C.H.	C.H.	N.C.H.
10	0.25 c.c.	0.2 c.c.	0.5 c.c.		Sl.H.	N.C.H.	N.C.H.	Sl.H.
11	0.25 c.c.	0.1 c.c.	0.5 c.c.		Sl.H.	Mod. H.	Mod. H.	Sl.H.
12	0.5 c.c.	——	——		0			
13	——	0.5 c.c.	——		0			
14	——	——	0.5 c.c.		0			

It had been previously determined that 0.0008 of a cubic centimeter of this amboceptor was the minimum hemolytic dose with one unit of complement. By working with dilutions so arranged that 0.25 of a cubic centimeter of dilution contained 0.0008 of a cubic centimeter of serum, another dilution slightly more, and a third slightly less than this amount, it was found that 1 to 350 was the proper dilution of amboceptor. This titration was repeated with several specimens of pooled complement until a constant optimum

[1] C.H. = complete hemolysis; N.C.H. = nearly complete hemolysis; Mod.H. = moderate hemolysis; Sl.H. = slight hemolysis; O = no hemolysis.

was determined. Two units of amboceptor diluted to 0.5 of a cubic centimeter with saline were used to sensitize each 0.5 of a cubic centimeter of the 5 per cent. cell suspension.

Complement.—The strength of the complement was determined each day. The previously determined optimum dilution of amboceptor was titrated against varying amounts of 10 per cent. complement (as shown in dilution 1 to 350, protocol I), and thus the minimum hemolytic dose of complement determined. The guinea pig serum was then diluted so that 0.5 of a cubic centimeter of the dilution represented two complement units. If hemolysis did not occur in tube 7 or went beyond tube 11, new complement was obtained. By accurately titrating the complement in this manner, the results of the fixation reactions from day to day are quite comparable.

Human Serum.—0.1 of a cubic centimeter of human serum inactivated one half hour at 56° C. was used in the test-tube; 0.2 of a cubic centimeter of the same serum was used in the serum anticomplementary control tube. The human serum was first pipetted into the tubes, then 0.4 of a cubic centimeter of saline was added to the test-tube, and 0.8 of a cubic centimeter of saline to the anticomplementary control tube. The complement was then added to all the tubes, and antigen to the test-tube. The volume of liquid in all the tubes was thus 1.5 cubic centimeters. Incubation was carried on in the water bath at 37° C. for one hour. The cells which had been sensitized by incubating 0.5 of a cubic centimeter of 5 per cent. cells with two amboceptor units for one half hour were then added to all the tubes, and incubation was carried on for another hour. Controls with positive serum, negative serum, and with antigen without serum were always employed.

Antigens.—Alcoholic extracts of the various organs were prepared as follows: The tissue, free from gross fat, was finely minced, weighed, and placed in absolute alcohol (Kahlbaum) in the proportion of one gram of tissue to ten cubic centimeters of alcohol. Extraction was carried on in the incubator at 37° C. for two to three weeks, the bottles being shaken daily. The extract was cooled to room temperature, filtered, and kept at room temperature as a stock solution. If a precipitate formed upon standing, the extract was

refiltered. The extraction was carried on in the incubator instead of by shaking because many laboratories are not equipped with a shaking machine, and it seemed desirable to adopt a technique for preparation of antigen which could be easily repeated.

The tissues selected for study were three human hearts, one beef heart, guinea pig hearts, guinea pig livers, and two non-syphilitic fetal livers. The guinea pig heart extract was made from a number of hearts, which were removed each day from the animals that had been killed for complement, weighed, minced, and placed with the requisite amount of alcohol in the common stock. This extract represented, therefore, the average extract of guinea pig hearts.

THE EFFECT OF VARYING CONCENTRATION OF CHOLESTERIN IN THE ALCOHOLIC TISSUE EXTRACT.

For this study four tissue extracts and a saturated alcoholic solution of a pure lipoid from fetal livers were used. The tissues represented in the different extracts were human heart, beef heart, guinea pig liver, and fetal liver. Saturated cholesterin solutions in the alcoholic extracts were prepared by adding known quantities of cholesterin to 100 cubic centimeters of the alcoholic extract, shaking for several hours, and then allowing to stand at 37° C. over night. Upon cooling to room temperature, the excess of cholesterin precipitated out. The amount of this precipitate was determined by filtering through weighed filters, drying and weighing the precipitate. The difference between the amount of cholesterin originally added and the weight of the filtrate showed the amount of cholesterin taken up by each extract. The amount of cholesterin taken up by the different crude extracts varied between 0.72 and 0.9 of a gram per 100 cubic centimeters. The exact amount is given in table I. The alcoholic solution of pure lipoid took up practically double this amount of cholesterin. Three fourths, one half, and one fourth saturated solutions were made by adding proper amounts of the original extract to the cholesterin-saturated extract.

Emulsions of the various dilutions were made by adding the extracts drop by drop, with constant shaking, to normal saline in the proportion of one part of the extract to five parts of saline, thus making a 1 in 6 emulsion. The emulsions were freshly prepared

each day in the same way. Sachs and Rondoni (8) showed that variations in the manner of mixing an alcoholic extract and saline varied the fixing power of the resulting mixture. Slow mixing of the alcoholic extract with the saline produced a heavy emulsion; rapid mixing, a colloidal suspension. The emulsion was more anticomplementary and fixed complement in smaller amounts in the presence of syphilitic serum. McIntosh and Fildes showed, however, that either slow or rapid mixing of their cholesterin-heart extract produced milky emulsions with equal fixing strength. We have repeated and confirmed this observation. If the extract is added slowly to the saline, a heavy emulsion forms at once; if added rapidly, the suspension is more opalescent at first, but upon standing becomes milky. After standing for one hour, both emulsions were tested for fixing power by using constant quantities of antigen against diminishing quantities of syphilitic serum. The point of disappearance of complete fixation was the same with both emulsions, thus proving that they had equal antigenic values.

With a constant method of emulsifying the antigen, the density of the emulsion was in direct proportion to the amount of cholesterin which had been added to the extract. In all instances, emulsion A (table I) was the heaviest, while emulsion E was opalescent.

The various emulsions from one extract were all tested for one property at the same time. The anticomplementary dose was first determined against two units of complement. This was done by increasing the amount of emulsion by steps of 0.1 of a cubic centimeter from 0.5 to 1.5 of a cubic centimeter. The addition of cholesterin increased the anticomplementary action of the extracts only slightly. With the heavier emulsions, the anticomplementary action was slightly more marked, but it began at practically the same point with all the emulsions of a given extract. The anticomplementary action of the heart extracts began at 1.2 cubic centimeters of the 1 in 6 emulsion, and at 1.4 cubic centimeters of the liver extracts.

The point at which fixation began with 0.1 of a cubic centimeter of serum from non-syphilitic patients was next determined. Serum from the same patients was used as much as possible with the various sets of extracts. The results of these experiments are summarized in table I and will be discussed later. Lastly, the smallest

amount of antigen which gave complete fixation with one fixing unit of syphilitic serum was determined. This fixing unit was established by titrating diminishing quantities of the serum with our old standard antigen. The amount of serum which just gave complete fixation was called a fixing unit. This amount was then diluted to 0.5 of a cubic centimeter with saline and tested against diminishing amounts of the various emulsions of the extract. The serum from both early and late stages of syphilis was tested with each set of emulsions.

Table I gives the point at which anticomplementary action of the emulsions began, the point of beginning fixation with non-syphilitic serum, and the point of complete fixation with syphilitic serum. In addition, the ratio of the fixing dose to the anticomplementary dose is given in per cent., or, in other words, the first two percentage columns indicate the proportion of the anticomplementary dose of antigen which showed beginning fixation with non-syphilitic serum, and the second two columns the proportion of anticomplementary dose which gave complete fixation with syphilitic serum. With the completely saturated, and three fourths and one half saturated cholesterin in the heart extracts, one unit of serum from patients with secondary syphilis gave complete fixation at points varying between 6 and 16 per cent. of the anticomplementary dose. One unit of serum from patients with late syphilis or tabes gave complete fixation with much smaller amounts of antigen; viz. between 1.6 and 6.6 per cent. With the one fourth saturation of cholesterin the point of fixation with negative serum rose between 15 and 30 per cent., while the curve with the serum from secondary syphilis rose from 8 per cent. for one half saturation to more than 60 per cent. for one fourth saturation of cholesterin. This falling off was less marked with serum from tertiary syphilis or tabes. The cholesterinized fetal liver extract reacted with negative serum and secondary syphilitic serum in much the same manner as did the heart extract, but with the serum from tabes a striking difference is noted. Fixation with this serum did not go to nearly so low a point with any of the liver extracts as it did with those from the hearts.[2] This would

[2] The results shown in table I were confirmed several times. Only examples with each class of serum, viz., non-syphilitic, early syphilitic, and late syphilitic, are given in order not to increase unnecessarily the size of the table.

TABLE I.

Alcoholic extract of		Human heart.						Beef heart.					
Saturation of cholesterin		Complete.	¾	½	¼	o		Complete.	¾	½	¼	o
Per cent. of cholesterin added	Diagnosis	0.9	0.67	0.45	0.22	0	Diagnosis	0.72	0.54	0.36	0.18	0
Emulsion		A	B	C	D	E		A	B	C	D	E
Anticomplementary dose in c.c.		1.2	1.2	1.2	1.2	1.2		1.2	1.2	1.2	1.2	1.2
Beginning fixation with 0.1 c.c. negative serum	Pneumonia	0.5	0.5	0.5	0.8	1	Pneumonia	0.6	0.5	0.6	1.0	1.1
Ratio of point of fixation with negative serum to anticomplementary dose in per cent.		41	41	41	66	80		50	41	51	80	90
Beginning fixation with 0.1 c.c. negative serum	Tuberculosis	0.6	0.5	0.6	0.8	0.8	Endocarditis	0.5	0.5	0.6	1.0	1.0
Ratio of point of fixation with negative serum to anticomplementary dose, in per cent.		50	41	50	66	80		41	41	50	80	80
Complete fixation with serum of early syphilis, in c.c.	Early secondary active[3]	0.2	0.2	0.1	0.7	0.9	Secondary active[4]	0.2	0.2	0.08	0.7	0.8
Ratio of point of fixation with fixing unit of serum from early syphilis to anticomplementary dose, in per cent.		16	16	8	60	80		16	16	6.6	60	80
Complete fixation with serum of late syphilis, in c.c.	Tabes[5]	0.06	0.06	0.08	0.1	0.7	Tertiary active[5]	0.02	0.02	0.04	0.1	0.7
Ratio of point of fixation with fixing unit of serum from late syphilis to anticomplementary dose, in per cent.		5	5	6.6	8.3	60		1.6	1.6	3.3	8.3	60

[3] Unit = 0.006 c.c.
[4] Unit = 0.007 c.c.
[5] Unit = 0.1 c.c.

Alcoholic extract of		Guinea pig liver.						Fetal liver.					
Saturation of cholesterin.		Complete.	¾	½	¼	0		Complete.	¾	½	¼	0
Per cent. of cholesterin added	Diagnosis	*0.8*	*0.6*	*0.4*	*0.2*	0	Diagnosis	*0.9*	*0.67*	*0.45*	*0.22*	0
Emulsion		A	B	C	D	E		A	B	C	D	E
Anticomplementary dose in c.c.		1.4	1.4	1.4	1.4	1.4		1.4	1.4	1.4	1.4	1.4
Beginning fixation with 0.1 c.c. negative serum	Pneumonia	0.5	0.6	0.8	1.2	1.2	Normal	0.6	0.6	0.8	0.8	1.2
Ratio of point of fixation with negative serum to anticomplementary dose, in per cent.		*35*	*43*	*57*	*90*	*90*		*43*	*43*	*57*	*57*	*90*
Beginning fixation with 0.1 c.c. negative serum	Endocarditis	0.5	0.6	0.9	1.2	1.2		——	—	—	—	—
Ratio of point of fixation with negative serum to anticomplementary dose, in per cent.		*35*	*43*	*64*	*90*	*90*		——	—	—	—	—
Complete fixation with serum of early syphilis, in c.c.	Secondary active[6]	0.2	0.3	0.6	1.1	1.2	Secondary latent[7]	0.2	0.2	0.1	0.2	0.5
Ratio of point of fixation with fixing unit of serum from early syphilis to anticomplementary dose, in per cent.		*14*	*21*	*43*	*80*	*90*		*14*	*14*	*7*	*14*	*50*
Complete fixation with serum of late syphilis, in c.c.	Tertiary active[7]	0.2	0.2	0.6	0.6	1.0	Tabes[7]	0.5	0.5	0.5	0.5	1.0
Ratio of point of fixation with fixing unit of serum from late syphilis to anticomplementary dose, in per cent.		*14*	*14*	*43*	*43*	*71*		*35*	*35*	*35*	*35*	*70*

[6] Unit = 0.013 c.c.

[7] Unit = 0.1 c.c.

seem to indicate that cholesterin-heart extracts are specially active with the serum of patients with long standing syphilis This view was substantiated in the second part of the present work.

The guinea pig liver extract acted somewhat differently than did the other extracts. The point of fixation with both normal and syphilitic serum fell off steadily as the concentration of cholesterin was diminished. With the simple alcoholic extracts of all the tissues (emulsion E) which contained no added cholesterin, the point of fixation with syphilitic serum approached so close to the point of fixation with negative serum and to the anticomplementary dose that for diagnostic purposes this form of emulsion had practically little value.

The result of the addition of cholesterin to a saturated alcoholic solution of pure lipoid prepared from fetal livers after Noguchi's method is not tabulated. These mixtures produced very heavy emulsions in salt solution. The point of fixation with normal serum was so close to the point of fixation with syphilitic serum that this form of extract could not be safely employed.

The results obtained with the two heart extracts have several points in common. Emulsions A, B, and C gave beginning fixation with negative serum at points between 40 and 50 per cent. of the anticomplementary dose. The fixing points with negative serum and with one unit of serum from secondary syphilis approached one another with emulsion D (one fourth saturation with cholesterin), and were practically the same with the simple alcoholic extract (emulsion E). With both heart extracts, as well as with the fetal liver extract, less of emulsion C than of emulsion A or B was required to give complete fixation with one unit of serum from early syphilis. The fixing point of all the cholesterin-heart extracts was low with the serum from late syphilis. The cholesterin fetal liver extracts gave fixation with normal serum and with serum from early syphilis in much the same amounts as did the cholesterin-heart extracts, but their fixing power with serum from late syphilis was much inferior to their fixing power with serum from secondary syphilis. The difference in the heart and liver extracts cannot be due to their original cholesterin content, because it was found that the heart extract contained 0.008 per cent. free cholesterin and 0.004

per cent. combined cholesterin, or a total of 0.012 per cent., while the liver extract contained 0.134 per cent. free cholesterin. If the cholesterin were alone responsible for the superiority of an extract, one would expect that the liver extract, which contained ten times as much cholesterin as the heart extract, would be the better, but this is not the case. It seems, therefore, that some other lipoid must be responsible for the superiority of the heart extract. The cholesterin guinea pig liver extracts were inferior to the other extracts, and the results obtained with them, compared with the results with two heart extracts or fetal liver extract, were so irregular that they were discarded in the subsequent work.

In the majority of trials, emulsion C was the most sensitive with serum from secondary syphilis, and was practically as sensitive as the stronger emulsions with the serum from late syphilis. In addition, its tendency to give non-specific fixation was somewhat less marked than were emulsions A and B. As previously mentioned, the density of the emulsions was in direct proportion to the amount of cholesterin it contained. Emulsions A and B were very milky, so that it was difficult to make immediate readings of the degree of hemolysis. As a result, the degree of partial hemolysis could not be determined until the following morning, when the non-hemolyzed cells had settled to the bottom of the tube. Emulsion C was not so milky, and with it immediate readings could be made with a fair degree of accuracy. In practically all respects emulsion C was superior to any of the other emulsions. Emulsion C consisted of equal parts of the saturated cholesterin extract and of the original extract. The amount of the added cholesterin in half saturation was between 0.36 and 0.45 per cent., or an average of slightly more than 0.4 per cent. The results of the first part of the work indicate that about 0.4 per cent. cholesterin is the optimum amount to add to crude alcoholic extracts of either hearts or human fetal livers. The effect of adding the cholesterin previously dissolved in alcohol was studied. Forty cubic centimeters of a 1 per cent. alcoholic solution of cholesterin were added to sixty cubic centimeters of the stock alcoholic extract of human heart C. The resulting mixture, therefore, contained 0.4 per cent. cholesterin. This was compared with the same alcoholic heart extract to which 0.4 per cent. cholesterin

had been added directly. The anticomplementary dose of 1 in 6 emulsions of the extracts was found to be 1.2 cubic centimeters for the cholesterin-heart extract and 1.1 cubic centimeters for the alcoholic cholesterin plus alcoholic heart extract. The cholesterin-heart extract gave slightly better fixation with syphilitic serum than did the alcoholic cholesterin plus alcoholic heart extract. The results of these experiments indicate that it was better to dissolve the cholesterin directly in the alcoholic extract of the heart. McIntosh and Fildes' cholesterin-heart extract was made by adding four parts of 1 per cent. alcoholic solution of cholesterin to five parts of heart extract which yielded a mixture containing 0.44 per cent. cholesterin; so the results obtained with their antigen and ours should be nearly comparable.

COMPARISON OF DIFFERENT EXTRACTS WITH THE SAME AMOUNT OF CHOLESTERIN ADDED TO EACH.

After it was decided that 0.4 per cent. cholesterin was the optimum amount to add to crude alcoholic extracts of the various organs, this amount of cholesterin was added to all the extracts at hand, and the resulting mixtures were compared for antigenic value. For this comparison we used extracts from three human hearts (C, D, and T), one beef heart (B), guinea pig hearts (G), and two fetal livers (L and K). As a standard for comparison we used three different antigens. One of them, antigen 29, was an alcoholic extract of fetal liver to which 0.25 per cent. cholesterin had been added. This had been in use for some time and had proven satisfactory according to our old standards. It was more sensitive than the antigens used in most other laboratories.[8] The other standard antigens were, first, an ether extract of dried human heart.[9] About two thirds of the sera were tested with this antigen. The remainder were tested with an alcoholic extract of syphilitic liver.[10] The combined results with these two antigens show that they were

[8] Dr. Jagle found it more sensitive than a standard antigen that was prepared in Sachs' laboratory in Frankfurt (personal communication).

[9] This was prepared by Tanenzen-Apotheke-R. Maertens, Berlin, after Fritz Lesser's formula.

[10] This was prepared by Gans, Frankfurt a/M, and standardized by Wassermann and Meier.

somewhat less active than our antigen 29. The 0.4 per cent. cholesterin heart and liver extracts were first tested for anticomplementary action; 1 in 6 emulsions were prepared and tested against two units of complement. It was found that the heart extracts were anticomplementary at about the same point, 1.2 cubic centimeters, and the liver extracts at 1.3 cubic centimeters. Three different strengths of emulsions of each extract in saline were tested for fixing power. These were 1 in 6, 1 in 10, and 1 in 30. 0.5 of a cubic centimeter of the 1 in 6 emulsion is about 40 per cent. of the anticomplementary dose. This amount was shown in the first part of the work to be the largest quantity that would not give nonspecific fixation. 0.5 of a cubic centimeter of 1 in 10 emulsion is 25 per cent., and the same amount of 1 in 30 is 8 per cent. of the anticomplementary dose. The first twenty-six sera were tested with all the emulsions. This number was sufficient to demonstrate that the cholesterin-liver extracts were not as sensitive as cholesterin-heart extracts, and that the three human heart extracts had practically the same fixing power. The comparison of two human heart extracts (D and T), and two fetal liver extracts (L and K) is given in the second part of table II. The remainder of the tests were carried on with cholesterinized extracts from one human heart (C), the beef heart (B), and the guinea pig hearts (G), as well as with the standard antigens 29, Lesser, and Wassermann. Most of the sera were from patients who had received more or less treatment with salvarsan and mercury. The few sera from untreated patients, both secondary and tertiary, gave complete fixation with all strengths of all the antigens. For purposes of comparison, the cases are divided into early and late syphilis. The early cases were all within three years from the time of infection,—most of them in the first year. The late cases were in the fourth year or later, and include cases of tabes dorsalis. There were thirty-five early cases and thirty-six late ones.

The results of the entire series are given in table II. The 1 in 6 emulsions of cholesterin-heart extracts gave a surprising number of strong positive reactions,—nearly three times as many as were obtained with the old standard antigens. As will be shown later, this 1 in 6 emulsion was probably too strong. The 1 in 10 emulsions

TABLE II.[11]

	Strength of emulsion.		1:6			1:10			1:30			Lesser.	Wassermann.	Total.	29		1:6				1:10				1:30			
	Extract.		C.	B.	G.	C.	B.	G.	C.	B.	G.						D.	T.	L.	K.	D.	T.	L.	K.	D.	T.	L.	K.
Early syphilis 35 cases	++	No.	20	18	21	10	8	12	8	8	8	4	1	5	7	18 cases	13	13	7	7	6	7	4	5	6	6	3	4
		Per cent.	57	51	60	29	23	34	23	23	23			14	20		72	72	40	40	33	40	22	28	33	33	17	22
	+	No.	5	5	4	7	4	6	1	1	2	1	1	2	1		2	2	4	5	1	3	2	1			1	1
		Per cent.	14	14	11	20	11	17	3	3	6			6	3		11	11	22	28	5	17	11	5			5	5
	±	No.	4	2	3	1	5	1	3	1	2	1	1	2	5		1			1	1	1	1	1		2	1	
		Per cent.	11	6	9	3	14	3	9	3	6			6	14		5			5	5	5	5	5		11	5	
	± (?)	No.	1	4	3	3	3	4	1	1	1	5		5	7		2	3	3	4	4	2	2	3	1	1		
		Per cent.	3	11	9	9	9	11	3	3	3			14	20		11	17	17	22	22	11	11	17	5	5		
	—	No.	5	6	4	14	15	12	22	24	22	11	10	21	14				4	1	6	5	9	8	11	9	13	13
		Per cent.	14	17	11	40	43	34	63	68	63			60	40				22	5	33	28	50	44	62	50	72	72
Late syphilis 36 cases	++	No.	23	19	23	18	17	18	13	13	14	2	5	7	6	8 cases	5	5	5	5	5	5	3	3	5	5	2	2
		Per cent.	61	53	61	50	47	50	36	36	40			20	17		63	63	63	63	63	63	37	37	63	63	25	25
	+	No.	5	8	5	5	2	5	2	2	2	2	3	5	3		1	1									2	2
		Per cent.	14	22	14	14	6	14	6	6	6			14	8		12	12									25	25
	±	No.	2	3	2	1	2	2	1	1			1	1	3		1	1		1	1							
		Per cent.	6	9	6	3	6	6	3	3				3	9		12	12		12	12							

[11] C, D, T = cholesterin-human heart extract; B = cholesterin-beef heart extract; G = cholesterin-guinea pig heart extract; L, K = cholesterin-fetal liver extract; Lesser = ether extract of human heart; Wassermann = alcoholic extract of syphilitic liver; 29 = cholesterin fetal liver extract (standard); ++ = complete inhibition of hemolysis; + = about 50 per cent. hemolysis; ± = about 75 per cent. hemolysis; ± (?) = nearly complete hemolysis; — = complete hemolysis.

Strength of emulsion.			1:6			1:10			1:30			Lesser.	Wasser-mann.	Total.	29	1:6				1:10				1:30			
Extract.			C.	B.	G.	C.	B.	G.	C.	B.	G.					D.	T.	L.	K.	D.	T.	L.	K.	D.	T.	L.	K.
Late syphilis 36 cases	± (?)	No.				1			2		1	1	1	2	5			2	1								
		Per cent.				3			6		3			6	14			25	12								
	−	No.	6	6	6	11	14	11	16	18	17	10	11	21	19	1	1	1	1	3	3	4	4	3	3	4	4
		Per cent.	17	17	17	30	40	30	44	50	47			60	53	12	12	12	12	37	37	50	50	37	37	50	50
Total early and late	++	No.	43	37	44	28	25	30	21	21	22			12	13	18	18	12	12	11	12	7	8	11	11	5	6
		Per cent.	60	52	62	40	34	42	30	30	31			17	19	70	70	46	46	42	46	27	33	42	42	20	23
	+	No.	10	13	9	12	8	11	3	3	4			7	4	3	3	4	5	1	3	2	1			3	3
		Per cent.	14	19	12	17	11	15	4	4	6			10	6	12	12	16	20	4	12	8	4			12	12
	±	No.	6	5	5	2	7	3	4	2	2			3	8	2	1		2	1	1	2	2		2	1	
		Per cent.	9	7	7	3	10	4	6	3	3			4	12	8	4		8	4	4	8	8		8	4	
	± (?)	No.	1	4	3	4	3	4	3	1	2			7	12	2	3	5	5	4	2	2	3	1	1		
		Per cent.	2	6	4	6	4	6	4	2	3			10	17	8	11	20	20	15	8	8	12	4	4		
	−	No.	11	12	10	25	29	23	38	42	39			42	33	1	1	5	2	9	8	13	13	14	12	17	17
		Per cent.	15	17	14	34	41	38	53	60	55			60	48	4	4	20	8	37	31	50	50	54	46	65	65

gave twice the number of strong positive reactions given by the old antigens. If we include + + and + reactions as strongly positive, the results are 57 per cent. with the human and guinea pig heart extracts, 45 per cent. with the beef heart, against 25 per cent. with antigen 29, and 27 per cent. for the combined results with the Lesser and Wassermann-Meier antigens. The 1 in 30 emulsions gave from 34 to 36 per cent. of strong positive reactions, which is an advantage of about 10 per cent. over the old antigens. Although the cholesterin-heart antigens were more sensitive in early syphilis, their superiority was more marked in late syphilis. With sera from early syphilis, the human heart antigen 1 in 10 gave 49 per cent. combined + + and + reactions, against 23 per cent. with antigen 29, and in late syphilis 64 per cent. of reactions of this strength with early syphilis and 42 per cent. with late syphilis. This increased sensitiveness with sera from late syphilis is a great advantage, for it is this class of cases in which the Wassermann reaction has such great diagnostic value.

The difference in cholesterin-heart and cholesterin-liver extracts with serum from early and late syphilis is well illustrated in the second half of the table. With serum from early syphilis and decreasing strength of antigens, that is, dilutions of 1 in 6, 1 in 10, and 1 in 30, the + + reactions with the best cholesterin-heart antigens were 72, 40, and 33 per cent., respectively, and with the cholesterin-liver antigen, 40, 28, and 22 per cent. In late syphilis the results with the cholesterin-liver antigens were 63, 37, and 25 per cent., while all dilutions of the heart-cholesterin antigens gave 63 per cent. positive reactions. Decreasing the strength of both kinds of antigen in secondary syphilis and of the cholesterin-liver antigen in tertiary syphilis decreased the number of positive reactions, while a similar diminution in strength of the cholesterin-heart antigen with sera from late syphilis had much less effect. This point was mentioned in the early part of the communication. A study of this rather striking difference between cholesterin-heart and cholesterin-liver antigens may throw some light on the nature of the Wassermann reaction. It should be emphasized that the sera were mostly from treated patients, so that the results do not indicate the percentage of positive reactions obtained with sera from early and

late untreated syphilis; all the sera from untreated patients gave strong positive reactions.

Human heart extracts were of practically the same strength. The guinea pig heart extract was very slightly stronger than the human heart extract. The beef heart extract, on the other hand, was decidedly inferior to the other heart extracts. The total + + and + reactions with 1 in 10 emulsions were 57 per cent. with the human and guinea pig heart extracts, against 45 per cent. with beef heart extract. As all the cholesterin-heart extracts were superior to similar preparations from human fetal livers, our results indicate that human or guinea pig hearts are the best tissues from which to prepare alcoholic extracts for reinforcement with cholesterin.

Twenty-two sera from patients in whom syphilis could be excluded were tested with all strengths of the three heart antigens, as well as with the standard antigens. Sixteen of these were entirely negative, and of these sixteen, twelve were also negative with 0.7 of a cubic centimeter of the 1 in 6 emulsion. Six of the twenty-two sera gave some degree of positive reaction with all of the 1 in 6 emulsions, three of these were + and three others ± (?). None of the twenty-two sera gave any inhibition with 1 in 10 or 1 in 30 emulsions. We feel that an antigen that gives any degree of positive reactions with 27 per cent. of non-syphilitic sera is too sensitive for diagnostic purposes. As all the non-syphilitic sera reacted negatively with 1 in 10 emulsions, we believe that this dilution is not too sensitive. Emulsions of this strength were one fourth of the anticomplementary dose, which should be well within the limits necessary to avoid non-specific fixation. McIntosh and Fildes state that one third of the anticomplementary dose of their cholesterin-heart antigen gave no non-specific reactions. They disregarded slight degrees of inhibition, calling such reactions negative. In view of the fact that such a large proportion of the non-syphilitic sera which we tested gave negative reactions with 1 in 6 emulsions, and that 15 per cent. of the sera from treated syphilitic patients also gave negative reactions with this strength of extract, it may be that syphilitic patients who are under treatment should be treated until their sera give negative reactions with 1 in 6 emulsions. This point

TABLE III.

Antigen.		29						C. 1:10						C. 1:6					
Amount of cerebrospinal fluid.		0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.05 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.05 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.05 c.c.
Syphilis	Cerebrospinal	++	++	+±	−	−	−	++	++	++	++	±	−	++	++	++	++	+	−
	Cerebrospinal	++	++	++	++	−	−	++	++	++	++	++	−	++	++	++	++	++	±
	Cerebrospinal	++	+±	±	−	−	−	++	++	++	++	±	−	++	++	++	++	+±	−
	Cerebrospinal	−	−	−	−	−	−	+±	+	−	−	−	−	++	+±	±	−	−	−
	Cerebrospinal	++	++	++	++	++	−	++	++	++	++	++	−	++	++	++	++	++	−
	Cerebral	−	−	−	−	−	−	++	++	++	+±	−	−	++	++	+	−	−	−
Tabes		++	++	++	++	−	−	++	++	++	++	+±	−	++	++	++	++	+±	−
Tabes		±	−	−	−	−	−	++	++	++	±	−	−	++	++	++	±	−	−
Tabes		++	++	+	±	−	−	++	++	++	++	−	−	++	++	++	+	−	−
Tabes		++	++	++	++	+	−	++	++	++	++	+±	−	++	++	++	++	±	−
Tabes		++	++	+	±	−	−	++	++	++	±	−	−	++	++	++	±	−	−
Tabo paralysis		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Summary	++	9	9	6	5	2	1	12	11	11	9	6	1	12	12	10	7	5	1
	+	0	0	2	0	1	0	0	1	0	0	1	0	0	0	1	1	1	0
	±	1	0	1	2	0	0	0	0	0	2	0	0	0	0	1	2	1	1
	−	2	3	3	5	9	11	0	0	1	1	5	11	0	0	0	2	5	10

can be decided only after prolonged observation of a series of treated patients.

The results of the examination of twelve cerebrospinal fluids is given in table III. In addition, fifteen cerebrospinal fluids from well treated tabetics or from patients in earlier stages of syphilis were negative with all the antigens. It has been found that the use of larger quantities of spinal fluid than serum will give a high per cent. of positive reactions if the fluids are from patients with syphilitic disease of the central nervous system. The quantitative reaction in the cerebrospinal fluid permits accurate estimation of the point at which fixation begins. The slight anticomplementary action of spinal fluid as compared with blood serum makes it a most suitable reagent for comparison of antigens. Table III, in which are presented the results obtained with positively reacting fluids, shows that the heart-cholesterin antigens gave complete fixation with from 0.1 to 0.3 of a cubic centimeter less fluid than did antigen 29. More interesting is the comparison of the 1 in 10 and 1 in 6 emulsions of the heart-cholesterin antigen. Although both emulsions gave positive fixations with 0.5 of a cubic centimeter of all the fluids, with 0.3, 0.2, and 0.1 of a cubic centimeter of fluid there were more complete fixations with the 1 in 10 emulsions than with the 1 in 6. We are unable to explain this paradox of more fixations with an apparently weaker antigen. The absence of any marked anticomplementary action on the part of cerebrospinal fluid seems to warrant the conclusion that the 1 in 10 emulsion has as good a specific action as the stronger emulsion. Compared with the results which we obtained with blood sera, one might be justified in concluding that the larger per cent. of positive reactions obtained with 1 in 6 emulsions with the serum was due to the more marked anticomplementary action of emulsions of this strength. The results with the spinal fluid indicate that the 1 in 10 emulsion gives the maximum specific fixation. We know that the stronger emulsion is much more anticomplementary, so that the larger number of positive reactions given by it, both with syphilitic and non-syphilitic sera, could be easily explained by the summation of anticomplementary action of the blood serum and of the strong emulsion. This hypothesis cannot be invoked to explain the superiority of the 1 in

10 emulsion of the cholesterin-heart antigen over antigen 29 or the syphilitic liver extract, for the anticomplementary dose of these two extracts was only twice the amount used in the tests, while the 1 in 10 emulsion of the cholesterin-heart antigen was one fourth of the anticomplementary amount. The superiority of the cholesterin-heart antigens must be due to the peculiar lipoids extracted from the heart muscle. The ease in securing human hearts, and the comparatively cheap method of extracting a lipoid of constant value renders these extracts peculiarly suitable. The addition of cholesterin in suitable quantities increases the antigenic value of these extracts so that they are superior to any other antigen that we have tried.

SUMMARY AND CONCLUSIONS.

1. The addition of cholesterin to an alcoholic extract of heart or fetal liver increases the antigenic value of the extracts in the Wassermann reaction.

2. The optimum amount of cholesterin to be added to heart extract or fetal liver extract was found to be 0.4 per cent.

3. Cholesterin-heart extracts are superior to cholesterin-liver extracts and to alcoholic extracts of syphilitic livers, as well as to ether extracts of dried hearts.

4. Cholesterin-heart extracts prepared from different human hearts are practically equal in anticomplementary and antigenic value. Similar extracts prepared from guinea pig hearts have the same antigenic value as those prepared from human hearts. Both the human heart and the guinea pig heart extracts are superior to beef heart extract when the same amount of cholesterin is added to each of the extracts.

5. In testing blood serum for diagnostic purposes, it is not safe to use more than one fourth of the anticomplementary dose of the 0.4 per cent. cholesterin heart extract. In the work here presented, this consisted of a 1 in 10 emulsion.

6. In testing cerebrospinal fluids, 1 in 10 emulsions give slightly better reactions with smaller quantities of the fluid than do 1 in 6 emulsions.

7. Because of the simple preparation, the superior antigenic prop-

erty, and the constant antigen value of cholesterin-heart extracts prepared from human hearts, we agree with McIntosh and Fildes that this form of extract fulfills the requirements of a standard antigen.

BIBLIOGRAPHY.

1. Lesser, F., *Berl. klin. Wchnschr.*, 1909, xlvi, 974.
2. Dembowski, H., *Deutsch. med. Wchnschr.*, 1911, xxxvii, 1651.
3. Noguchi, H., *Jour. Exper. Med.*, 1909, xi, 84.
4. Browning, C. H., and McKenzie, I., Diagnosis and Treatment of Syphilis, Philadelphia, 1912.
5. Sachs, H., *Berl. klin. Wchnschr.*, 1911, xlviii, 2066.
6. McIntosh, J., and Fildes, P., *Ztschr. f. Chemotherap., Orig.*, 1912, i, 79.
7. Wassermann, A., Neisser, A., and Bruck, C., *Deutsch. med. Wchnschr.*, 1906, xxxii, 745.
8. Sachs, H., and Rondoni, P., *Ztschr. f. Immunitätsforsch., Orig.*, 1909, i, 132.

MALARIAL PIGMENT (HEMATIN) AS AN ACTIVE FACTOR IN THE PRODUCTION OF THE BLOOD PICTURE OF MALARIA.*

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In a recent article[1] the author submitted evidence to show that hematin intoxication in the rabbit was characterized by a paroxysm analogous to that of malaria, and a study of the effect of this pigment upon the blood is offered as further evidence of the rôle that hematin plays in malaria. While particular attention has here been directed to the formed elements of the blood, other important effects have been noted, such as the effect upon the coagulation time and the bleeding time.

The hematin, the hematin solutions, the doses, and the technique used in these experiments were identical with those used in previous experiments. In fact, many of the observations upon which this report is based were made in the course of experiments described in my former article. The experiments have been graded so as to determine the effect of a single injection of various amounts of alkaline hematin, and finally to follow the changes produced by injections repeated at various intervals over a period of days or even weeks. All phases of the experiments were controlled in two ways. One series of controls was made by injecting rabbits with the same amount of the solvent for the hematin (1 or 2 per cent. bicarbonate of soda dissolved in 0.85 per cent. sodium chloride solution) per kilo of body weight, and a second series of controls was made by first injecting the alkaline salt solution, and, after the

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[1] Brown, W. H., *Jour. Exper. Med.*, 1912, xv, 579.

animal's blood had returned to normal, injecting the hematin solution. This procedure was followed with only a few animals.

The Effect of Hematin upon the Red Blood Corpuscles.—The red blood corpuscles, although decidedly less subject to the toxic influence of hematin than either the leucocytes or the platelets, may be markedly reduced in numbers by the intravenous injection of hematin. A single injection of less than fifteen milligrams of hematin per kilo of body weight seldom produces any demonstrable effect upon the red cell count. If there is a decrease, regeneration is sufficiently rapid to obscure the change and this is probably the case. An injection of as much as twenty milligrams of hematin per kilo, however, usually shows, within a few hours, a decrease of as much as 1,000,000 cells, and frequently more. This decrease is still only transient although a reduction may be evident for as long as twenty-four hours.

The daily repetition of a dose of even ten milligrams of hematin per kilo will ultimately produce a definite anemia, and the larger the dose the more marked is the anemia. In well marked anemia the cells are irregular in size, the color is variable, and immature cells are present. The most common of these is the young basophilic cell, although nucleated red cells may be present in considerable numbers. Such an instance is illustrated by the following experiment:

Rabbit 5.—Albino, female; weight, 2.85 kilos. Within seven days this animal received eleven injections averaging 10 mg. of hematin per kilo of body weight. The red cells showed the following changes:

May 2, 1911. Red blood corpuscles, 7,596,000; nucleated red cells, 3 (normoblasts).

May 9. Red blood corpuscles, 3,200,000; nucleated red cells, 179; normoblasts, 153; intermediates, 21; megaloblasts, 5.

This experiment is cited merely to show to what degree the destruction of red cells can be pushed under favorable circumstances. The usual reduction, however, does not exceed one third of the normal count and nucleated cells are comparatively few.

As regards the hemoglobin, with single injections and slight reduction in the red cells, the hemoglobin index (Sahli) is not altered from the normal. With more pronounced destruction of red cells, and especially with the influx of immature cells into the circulation,

the hemoglobin index is usually reduced, but it may be normal or rarely increased above the normal. In some of these cases it is possible that hematin still in the circulation has influenced the hemoglobin determinations.

The anemia produced by the intravenous injection of alkaline hematin is due entirely to the hematin, as the alkaline salt solution has been found entirely incapable of producing the slightest degree of anemia, even when used in much larger amounts than were employed with the hematin. In fact, the only effect upon the red blood corpuscles, observed in the control animals, was in the opposite direction. Most of the animals showed no appreciable effect, while a few showed a very definite increase in the red cell count with a corresponding increase in the percentage of hemoglobin.

The mechanism of destruction of the red cells is by no means clear. *In vitro,* the alkaline hematin solution produces no hemolysis and this would be expected on account of the hypertonicity of the solution. In the animal body, on the contrary, some degree of hemolysis has been detected in many instances. The hemolysis, however, does not depend entirely upon the amount of hematin injected, as marked hemoglobinemia was noted in one case after an injection of ten milligrams of hematin per kilo of body weight, and was very slight or not demonstrable in several other instances where the dose of hematin was as much as twenty milligrams per kilo. The evidence at hand is not sufficient to warrant an expression of opinion as to the factors that determine the hemolytic action of hematin.

Microscopic study of the tissues shows that red cells are also destroyed intact as numbers of these cells are found included within phagocytic cells.

The Effect upon the Leucocytes.—The leucocytes, unlike the red cells, are markedly effected by simple alkaline salt solutions as well as by solutions of hematin, and this circumstance has added to the difficulties of establishing the leucocyte picture of hematin intoxication. The intravenous injection of alkaline salt solution produces a rapid decrease in the total number of leucocytes. Within three to five hours the count has again risen to normal, the rise continuing to a definite leucocytosis which lasts only a few hours and then

slowly falls to normal. A count taken twenty-four hours after the injection practically always shows a normal number of leucocytes. Differential counts (500 cells) show that all types of cells participate in these changes to some degree. The polymorphonuclear amphophile, however, is the cell that is most influenced. These cells show an immediate absolute reduction and frequently a relative reduction as well; they then increase rapidly so that their actual number may be well above the normal, while all other cells are still decreasing or at their lowest point; then follows a gradual fall to normal. Next to the polymorphonuclear cells, the large mononuclear and transitional group shows the most pronounced effect, an effect that is characterized by a rapid decrease with a slow recovery which continues well above the normal, both as regards percentage and actual numbers, but rarely exceeds 8 to 10 per cent. The small mononuclears decrease but slowly and regenerate even more slowly. The eosinophiles and basophiles are inconstant in their behavior, the former being but little affected while the latter may show marked fluctuations, characterized by an initial decrease and a marked subsequent increase.

By repeating the injections of alkaline salt solution daily, for a week or more, this picture is altered but little. The most important change is to be found in an increase of the mononuclear cells at the expense of the polymorphonuclears. The large mononuclears under these conditions may reach 12 to 14 per cent.

When the effect upon the leucocytes of a single dose of ten to fifteen milligrams of hematin per kilo is compared with the effect of an equivalent volume of alkaline salt solution, the differences that can be detected are but slight. To facilitate comparison the following abbreviated protocol and series of counts from one of my experiments are given.

Rabbit 48.—Belgian, male; weight, 2 kilos.

November 13, 1912. 9.30 A. M. White blood corpuscles, 6,600. Injected 6.6 c.c. of 2 per cent. sodium bicarbonate in 0.85 per cent. sodium chloride solution. The leucocytes were counted at intervals of one, three, five, and twenty-six hours.

November 20. 9.30 A. M. White blood corpuscles, 8,800. Injected 6.6 c.c. of hematin solution (10 mg. of hematin per kilo). The leucocytes were counted at intervals of one, three, five, and twenty-six hours. Complete counts are given in table I.

TABLE I.

The Leucocyte Reactions Produced by a Single Dose of Ten Milligrams of Hematin per Kilo of Body Weight and by a Corresponding Volume of Alkaline Salt Solution.

Time.	Leucocyte count.	Polymorphonuclear cells.		Small mononuclear cells.		Large mononuclear cells.		Eosinophiles.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Nov. 13, 1912, 9.30 A.M.	6,600	54.0	3,564	32.8	2,164	6.4	422	0.8	52	6.0	396
10.30 A.M.	3,600	56.0	2,016	38.4	1,382	1.6	57	0.4	14	3.6	129
12.30 P.M.	5,800	82.0	4,756	15.6	904	2.0	116	0.0	0	0.4	23
2.30 P.M.	8,600	78.8	6,776	15.2	1,307	4.4	378	0.0	0	1.6	137
Nov. 14, 11.30 A.M.	6,600	57.2	3,775	17.4	1,148	9.4	620	0.4	26	15.6	1,029
Nov. 20, 9.30 A.M.	8,800	45.6	4,012	39.2	3,449	7.2	633	1.6	140	6.4	563
10.30 A.M.	5,800	25.5	1,479	71.0	4,118	2.5	145	1.0	58	0.0	0
12.30 P.M.	13,000	75.2	9,776	21.2	2,756	2.4	312	0.0	0	1.2	156
2.30 P.M.	9,200	77.6	7,139	17.2	1,582	3.6	331	0.4	36	1.2	110
Nov. 21, 11.30 A.M.	6,800	26.4	1,795	53.2	3,617	15.2	1,033	0.0	0	5.2	353

While many differences in the two series of counts in table I are apparent, the only feature to which I attach any importance is the relatively larger number of mononuclear cells, including large and small mononuclears, in the hematin series as compared with the control series at the end of the twenty-six hour periods. This difference has been practically constant under these conditions, but an almost equally marked increase in mononuclear cells may be encountered after repeated injections of alkaline salt solution over a period of several days. The reduction in the total leucocyte count from 8,800 to 6,800, as shown in the hematin counts, is misleading, as I have found an increase in the leucocyte count more common than a decrease. This feature of the hematin reaction will be considered later.

The production of a chronic intoxication by daily injections of small doses of hematin for a week or more brings out distinctly

the resemblance of the leucocyte picture of hematin intoxication to that of malaria. Following each dose of hematin there is a cycle of changes, such as is illustrated in table I, with the mononuclear cells dominating the picture more and more; especially is this true of the large mononuclears which gradually increase until they constitute from 18 to 25 per cent. of a leucocyte count that is normal, slightly increased, or rarely reduced. The tendency to a slight leucocytosis is still the one striking discrepancy between the two pictures. Pigmented macrophages are frequently present in large numbers and have been found in the blood as much as seventy-two hours after an injection of hematin. The polymorphonuclear leucocytes may also show phagocytosis although this is more common in extreme intoxications.

A typical example of the effect of chronic hematin intoxication upon the leucocytes is presented in table II, which contains the normal and final leucocyte counts made upon rabbit 15.

Rabbit 15.—Grey and white, male; weight, 1.6 kilos.

January 16, 1912. White blood corpuscles, 8,300.

This animal received 10 mg. of hematin per kilo of body weight on ten successive days.

January 27. White blood corpuscles, 11,800. The differential counts are shown in table II.

TABLE II.

The Leucocyte Reaction in Chronic Hematin Intoxication.

Time.	Leucocyte count.	Polymorphonuclear cells.		Small mononuclear cells.		Large mononuclear cells.		Eosinophiles.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Jan. 16, 1912, 8.30 A.M.	8,300	42.8	3,552	48.4	4,017	5.6	465	1.4	116	1.8	149
Jan. 27, 9.00 A.M.	11,800	23.8	2,808	50.2	5,924	22.2	2,620	0.4	47	3.4	401

If the conditions existing in pernicious forms of malaria be simulated by the injection of as much as twenty milligrams of hematin per kilo of body weight, and one or two injections be given daily,[2]

[2] Unless the animal is in the best condition it can not survive this dosage of hematin. The first injection will prove fatal to a few rabbits and the number of deaths will increase with successive injections, especially if the injections are given less than ten to twelve hours apart.

within a few days the animal will develop a marked leucocytosis. The polymorphonuclear amphophiles show a relative increase, the percentage of small mononuclears is decreased or normal, while the percentage of large mononuclears is normal or increased. Both the eosinophiles and basophiles are relatively decreased under these conditions. The most striking feature of the count is the high percentage of large mononuclear cells. While the percentage increase in these cells is not so great as in chronic intoxications it not infrequently reaches 12 or 14 per cent. of the total count. The normal and the final counts in an experiment of this character are given to illustrate this point.

Rabbit 19.—Grey, female; weight, 1.7 kilos.

March 18, 1912. White blood corpuscles, 11,400. Seven injections of 25 mg. of hematin, per kilo of body weight, were given between March 18 and 21, inclusive.

March 22. White blood corpuscles, 52,600.

TABLE III.

The Leucocyte Reaction in a Case of Extreme Hematin Intoxication.

Time.	Leucocyte count.	Polymorphonuclear cells.		Small mononuclear cells.		Large mononuclear cells.		Eosinophiles.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
March 18, 9.00 A.M.	11,400	36.8	4,195	47.6	5,426	7.0	798	3.0	342	5.6	638
March 22, 9.00 A.M.	52,600	62.2	32,710	20.6	10,835	15.0	7,890	1.0	526	1.2	631

It will be noted that the increase in large mononuclear leucocytes, as shown in table III, is relatively greater than the increase in polymorphonuclear cells. This is not usually the case and is, therefore, to be regarded as an extreme example of the reaction of the large mononuclear leucocytes to hematin.

On reviewing the changes in the leucocytes induced by the injection of alkaline hematin, the increase in the total count of these cells with the lesser degrees of intoxication seems to be the feature most at variance with the leucocyte picture of malaria A partial explanation of this difference is to be found in the fact that alkaline hematin is a strong local irritant and that it is extremely difficult

to inject into an ear vein, even in dilute solution, without exciting a local inflammatory reaction, while thrombosis of the vein is certain to follow a limited number of injections into the same vessel. Although all animals that showed any considerable inflammatory reaction have been discarded, it seems possible that with the strength of alkaline hematin that it has been necessary to use, the irritant action has not been without effect. The degree of this effect is impossible of estimation.

As regards the most characteristic feature of the leucocyte reaction,—the increase in large mononuclear cells,—a portion of this increase is certainly attributable to the alkaline salt solution used as the solvent for hematin, but, when this is deducted, I believe there is still a substantial margin that can be accounted for only by the action of the hematin.

Platelets, Coagulation, and Bleeding Time.—The most striking effect of hematin upon the blood is its destruction of the platelets and consequent prolongation of the bleeding time. While the normal number of platelets in the rabbit varies between rather wide limits, it is usually between 600,000 and 800,000, estimated by the method of Wright and Kinnicutt,[3] and in a given animal it remains fairly constant from day to day. Following the injection of even a small dose of hematin, there is an immediate reduction in the number of platelets and the count continues to decrease for several hours. Large doses of hematin produce a correspondingly greater destruction of platelets which may exceed one half the total platelet count. If no further injections are given, the count will return to normal, or above, within twenty-four to forty-eight hours. Daily repetition of the injections of hematin, however, will greatly reduce the platelet count,—usually not lower than about one fourth of the normal count. A single dose of hematin sufficiently large to produce a greater reduction, or the repetition of smaller doses sufficiently often to accomplish the same result, are apt to prove fatal. In several instances, however, I have recorded greater reductions than this, the greatest being a reduction from 928,000 to 92,000. The series of counts in table IV is intended to illustrate the essential features of the effect of hematin upon the platelets as compared with the effect of alkaline salt solution.

[3] Wright, J. H., and Kinnicutt, R., *Jour. Am. Med. Assn.*, 1911, lvi, 1457.

TABLE IV.

The Effect of Hematin upon the Platelet Count.

Time after injection.	No. 34. 25 mg. of hematin per kilo.	No. 31. An equivalent volume of alkaline salt solution.
Normal	616,000	560,000
1 hr.	192,000	598,000
3 hrs.	232,000	740,000
5 hrs.	156,000	646,000
24 hrs.	312,000	608,000
48 hrs.	480,000	594,000
75 hrs.	788,000	622,000

In connection with the destruction of the platelets, a tendency to persistent and even profuse hemorrhage is of frequent occurrence. A series of observations made on the coagulation time with the Boggs coagulometer and on the bleeding time by the method of Duke[4] may be reported briefly. It was found that the ultimate effect of hematin was to prolong both the coagulation time and the bleeding time. The longest coagulation time recorded with the Boggs instrument was seventeen minutes, while eight to twelve minutes were not uncommon. In several instances, however, blood drawn in a test-tube remained permanently unclotted. The bleeding time is apparently much more affected. The ear vessels are usually abnormally constricted but if a normal degree of dilatation be procured profuse hemorrhage occurs from small cuts and bleeding continues for hours. In several extreme cases it was necessary to place a clamp on the ear after bleeding had continued two or three hours with no indication of cessation.

These phases of the action of hematin are apparently not influenced in the slightest degree by the presence of the alkaline salt solution. As regards the platelets, control experiments show almost uniformly an increase in the platelet count (table IV) after the injection of alkaline salt solution. Not a single instance of a definite decrease in the platelets has been observed. And no effect of any

[4] Duke, W. W., *Jour. Am. Med. Assn.*, 1910, lv, 1185.

kind has been noted upon either the coagulation or the bleeding time following the injection of alkaline salt solution.

DISCUSSION.

Comparing the various phases of the effect of alkaline hematin upon the blood of the rabbit with the blood in malarial fevers it can not be claimed that there is an absolute identity and yet there are no very great differences. The characteristic anemia of malaria has been ascribed to several accessory causes apart from the destruction of the red cells by the parasite developing within them. Prominent among these accessory causes has been the theory of a circulating toxin, destructive to the red cells. Hematin fulfills the essential conditions for such a circulating toxin, and it is only remarkable that this pigment should cause so pronounced an anemia as it has been found capable of producing. In this connection, it does not seem amiss again to call attention to the fact that the hemoglobin converted to hematin by the malarial parasite is not readily available for the regeneration of red cells, and the blood in this manner is rapidly depleted of hemoglobin iron. This condition undoubtedly contributes to the perpetuation of the malarial anemia.

Concerning the influence of hematin upon the leucocytes of malaria, one can speak with less assurance. In the first place, the possible normal variations of these cells in the rabbit are very great. Added to this is the influence of alkaline salt solution, which is difficult to estimate, and finally the difficulty of adjusting the dose of hematin so as to produce desired degrees of intoxication. Therefore only broad generalizations seem to be permissible. The leucocytosis of hematin poisoning is in sharp contrast to the leucopenia of the milder forms of malaria, but it coincides with the leucocytosis of the pernicious forms. The increase in the large mononuclear leucocytes, which is so characteristic of malarial blood, is also a prominent feature of the blood of hematin intoxication. Although the percentage of these cells is probably not as high as in malaria, this would hardly be expected as there are certainly other causes that contribute to the increase in large mononuclear leucocytes Finally, the general cycle of changes produced by an injection of hematin is, at least qualitatively, analogous to changes occurring in the blood with the paroxysm of malaria. Altogether,

it would seem that hematin must play some part in the determination of the leucocyte picture of malaria.

Lastly, the destructive action of hematin upon the platelets and the consequent tendency to hemorrhage is not without a parallel in malaria. The platelets are probably the only bodies in the blood whose numbers in malaria are influenced solely by the circulating toxin of the disease, and it is of especial significance, therefore, that the platelets are reduced both in malaria and in hematin intoxication. The tendency to hemorrhage produced by hematin has its counterpart in the well recognized class of hemorrhagic cases of pernicious malaria. Here again the circulating hematin probably plays the important rôle in the production of the hemorrhagic condition. I do not wish to create the impression, however, that hemorrhages in malaria, or in hematin poisoning, are due solely to the destruction of platelets and prolongation of the bleeding time. This is certainly not the case in hematin poisoning in which profuse hemorrhage into the peritoneal cavity is of frequent occurrence in acutely fatal poisoning.

The pigment, hematin, must be regarded as an active factor in the production of many, if not all, of the important changes in the blood that characterize the various forms of malaria.

SUMMARY.

1. Intravenous injections of alkaline hematin in the rabbit produce an anemia the severity of which is proportional to the amount of hematin injected and the susceptibility of the animal.

2. Hemoglobinemia is an occasional consequence of hematin poisoning.

3. The leucocytes in hematin intoxication are usually increased in number and are always characterized by a high percentage of large mononuclear cells and by pigmented phagocytes.

4. The platelets are markedly reduced by alkaline hematin and ultimately a prolongation of the coagulation time of the blood and of the bleeding time results.

5. The anemia, the hemoglobinemia, the high percentage of large mononuclear leucocytes, the destruction of platelets, and the tendency to hemorrhage in malaria are all influenced by the malarial pigment, hematin.

THE EFFECT OF HEMATIN ON THE CIRCULATION AND RESPIRATION.*

By WADE H. BROWN, M.D., and A. S. LOEVENHART, M.D.

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Plates 2 to 5.

In the course of investigations which one of us has been conducting upon the toxic manifestations of hematin when introduced into the animal circulation, the nature of the effects elicited with both sublethal and lethal doses of alkaline hematin was such as to indicate clearly that we were dealing with a substance that possessed a definite action upon the circulation and the respiration. Some phases of the toxic action of hematin in the rabbit have been described in detail in another paper,[1] and it is sufficient here to point out that the most characteristic clinical effect of the injection of small doses of hematin was found to be the production of a paroxysm similar to the malarial paroxysm in man. During the cold stage of this paroxysm, a marked constriction of the skin vessels occurs, seen especially in the ears, with a lowering of the surface temperature. This vasoconstriction gives place to an equally marked dilatation of the skin vessels and elevation of surface temperature with the onset of the hot stage of the paroxysm. It was further noted that, in those animals which succumbed to the toxic action of hematin within a short time after its administration, respiratory distress, marked prostration, extreme pallor, convulsions, and failure of the respiration were almost constant symptoms. In these animals there was usually found, post mortem, a pronounced splanchnic congestion with hemorrhage into the viscera and into the peritoneal cavity.

Accordingly, a study of the physiological action of hematin in

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[1] Brown, W. H., *Jour. Exper. Med.*, 1912, xv, 579.

alkaline solution offered a hope for a tangible explanation of these and other phenomena, many of which are characteristic alike of both hematin intoxication and human malaria.

The hematin used in these experiments was prepared from ox blood; the mode of preparation of the hematin and the alkaline solution was the same as that employed in experiments previously reported.[2] The solution used in all cases was formed of an 0.85 per cent. sodium chloride solution containing 2 per cent. sodium bicarbonate and either four or five milligrams of hematin per cubic centimeter. The effects obtained by the injection of this solution were controlled by injections of the same preparation of alkaline salt solution as that used in the preparation of the hematin solution. The injections were all made into the femoral vein.

Action on the Circulation.—The effect of hematin upon the blood pressure and upon the distribution of the blood in the body is very pronounced. Following the injection of doses of less than six milligrams of hematin per kilo of body weight in the cat or in the dog, a slight rise of the carotid pressure may occur. The injection of six to eighteen milligrams per kilo, however, produces a great fall of blood pressure in the cat which reaches a maximum in from one to five minutes and remains below normal from twelve to thirty minutes, or even longer in exceptional cases. In the dog the fall in pressure is usually not so great, but the duration of the effect is usually more prolonged than in the cat. The extent to which hematin may lower the blood pressure is shown in figures 1 and 2. Both of these tracings are from the cat and represent respectively effects of doses of eighteen and twenty-two milligrams of hematin per kilo of body weight.

On plethysmographing a loop of intestine and one hind leg simultaneously, and recording alterations in volume with piston recorders, it was found that prior to the fall of blood pressure there occurs a dilatation of the intestinal vessels which begins before the blood pressure starts to fall and continues for a long time. In one experiment the blood pressure remained below normal for about twenty

[2] Brown, W. H., *loc. cit.*

minutes, when other procedures interrupted the record. In several of the experiments the intestinal dilatation was of an extreme grade. During the fall of blood pressure the vessels of the leg constrict markedly. This compensatory action is not so perfect in the cat, in which the dilatation of the splanchnic vessels is less marked, and the fall of blood pressure is more marked than in the dog. In one experiment the cutaneous constriction started later than the intestinal dilatation, which would suggest that the constriction may be due, in part, at least, to the depletion of the cutaneous vessels to supply the engorged splanchnic vessels. We have concluded, however, that the cutaneous constriction is active for the reasons which follow.

In one experiment a slight rise of blood pressure followed the injection of hematin although the injection caused splanchnic dilatation. From our observations on the heart it is improbable that this rise of blood pressure was due to increased cardiac output, and in this case the constriction of the cutaneous vessels would seem to have more than compensated for the splanchnic dilatation. Unfortunately, there was no plethysmographic tracing from the leg in this experiment.

Figures 1 and 2 show typical tracings of the splanchnic dilatation and its relation to the blood pressure in the cat, while figure 3 shows plethysmographic tracings of the intestine and leg and the carotid pressure of the dog.

The splanchnic dilatation seems to be due almost entirely to the depression of the vasomotor center, since section of both the splanchnic nerves usually prevents entirely the dilatation and greatly reduces or entirely removes the blood pressure-lowering action of hematin. In fact, after cutting the splanchnics, we have noted a rise of blood pressure which further indicates that the cutaneous constriction is active rather than passive to the splanchnic engorgement. In one experiment a distinct but rather small splanchnic dilatation together with a very slight fall of blood pressure followed the injection of hematin after the splanchnics were cut. This single experiment indicates that a small fraction of the dilating effect of the drug is peripheral.

Action on the Heart.—The only definite effect of hematin upon the rate of heart-beat that we have noted is a slowing. In a few instances where doses of less than six milligrams of hematin per kilo of body weight were injected a transient acceleration of a few beats per minute occurred. Although the degree of retardation of the heart action is extremely variable, within certain limits it is proportional to the size of the dose of hematin. This is especially noticeable in the cat in which a dose of twelve to eighteen milligrams per kilo almost invariably produces a typical vagal pulse (figures 1 and 2). The same effect is less easily produced in the dog. As no slowing of the pulse rate occurs after the administration of atropin or section of the vagi, we may conclude that the slowing is due to stimulation of the cardio-inhibitory center.

An extreme irregularity in the amplitude and rhythm of the heart-beat occurred in many of our experiments on the cat but rarely in experiments on the dog (figure 2).

One experiment was performed with the heart in a plethysmograph and the changes in the heart volume were recorded by means of a piston recorder. The injection of hematin was followed by a marked loss of tone in both systole and diastole and a great decrease in the cardiac output. This reaches a maximum within about one minute and then the tone of the heart gradually returns until it becomes greater than the normal although the output remains diminished (figure 4). The heart, therefore, plays a part in the blood pressure-lowering action of hematin. The relative parts played by the splanchnic dilatation and the decreased cardiac output probably vary greatly in different experiments.

Action on the Respiration.—The primary effect of the large doses in which we employed hematin resulted frequently in an increased rate, but rarely in an increased amplitude of the respirations, a decreased amplitude being much more frequent. It seems probable from our records, however, that had we used smaller doses a stimulation of the respiration would have resulted. The larger doses of hematin produce marked depression of the respiration. In several of our experiments, especially on the cat, the respirations rapidly diminished in size until there occurred a complete cessation of the

respiration. This period of complete stoppage lasts thirty seconds or longer and is terminated spontaneously by very small respirations which gradually increase in size until the normal is reached. The rate is also slow, following the paralysis of the respiration, and this likewise increases gradually to the normal (figures 1 and 2). Death under hematin is due to paralysis of the respiration, the heart continuing to beat for some time after the respiration ceases.

CONCLUSIONS.

1. Doses of less than six milligrams of hematin and, under certain conditions, larger doses may cause a slight rise of blood pressure.

2. Large doses of hematin cause a profound and prolonged fall of blood pressure. The principal factor in this fall of blood pressure is the marked dilatation of the splanchnic vessels. The splanchnic dilatation either does not occur at all or but very slightly if the splanchnic nerves are cut. The splanchnic dilatation is partly compensated for by a marked constriction of cutaneous vessels and it seems probable that the cutaneous constriction is active and not simply passive to the splanchnic engorgement.

3. Hematin acts upon the cardio-inhibitory center causing a marked slowing of the rate of heart-beat, and in large doses produces a typical vagal pulse or even marked irregularities in the amplitude and rhythm of the pulse. Under hematin the heart at first shows great loss of tone but later the tone increases beyond the normal. The cardiac output for a time is greatly diminished.

4. In the large doses employed by us, hematin depresses the respiratory center and death under hematin is due to paralysis of this center.

EXPLANATION OF PLATES.

PLATE 2.

FIG. 1. Cat; weight, 2.8 kilos. Two injections of 5 c.c. of hematin (9 mg. of hematin per kilo) were given one and one half minutes apart. The second injection was given at 7. Read from left to right.

PLATE 3.

FIG. 2. Cat; weight, 3.4 kilos. Three injections of 5 c.c. of hematin (7.3 mg. of hematin per kilo) were given two and one half minutes apart, the third injection being given at 5. Read from left to right.

PLATE 4.

FIG. 3. Dog; weight, 11.5 kilos. Four injections of 3.5 mg. of hematin per kilo were given in rapid succession. Read from left to right.

PLATE 5.

FIG. 4. Cat; weight, 2.3 kilos. 5 c.c., or 8.5 mg. of hematin per kilo, were given at 2 and repeated at 3. Read from left to right.

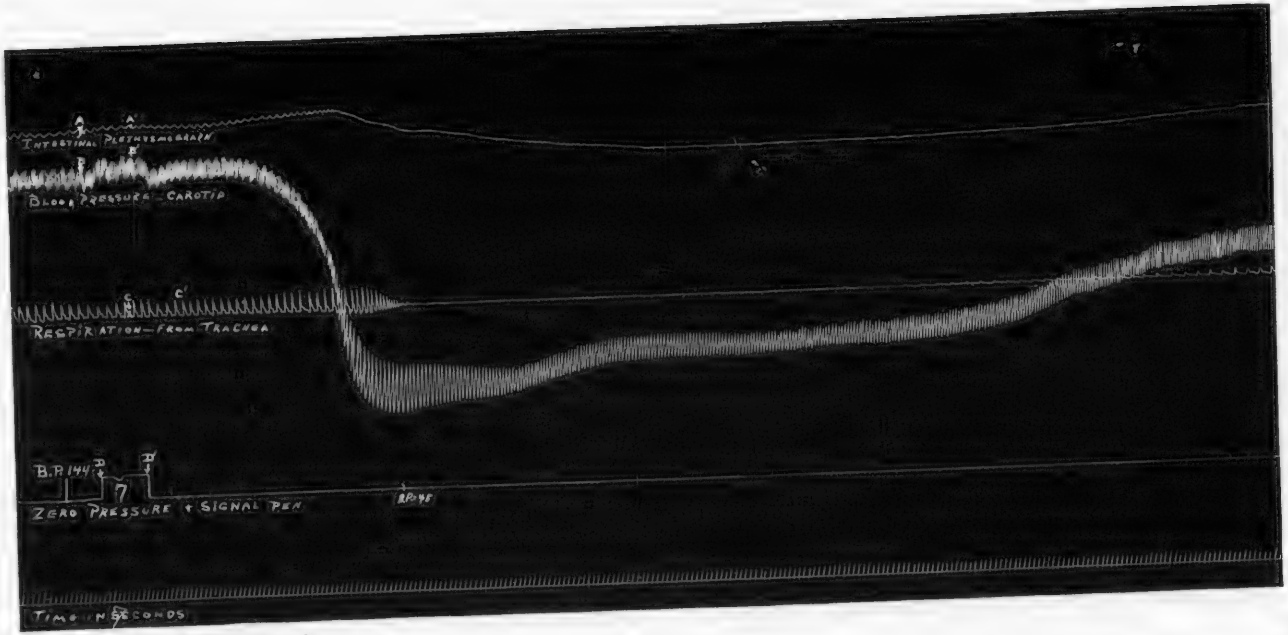


FIG. 1.

(Brown and Loevenhart: Effect of Hematin.)

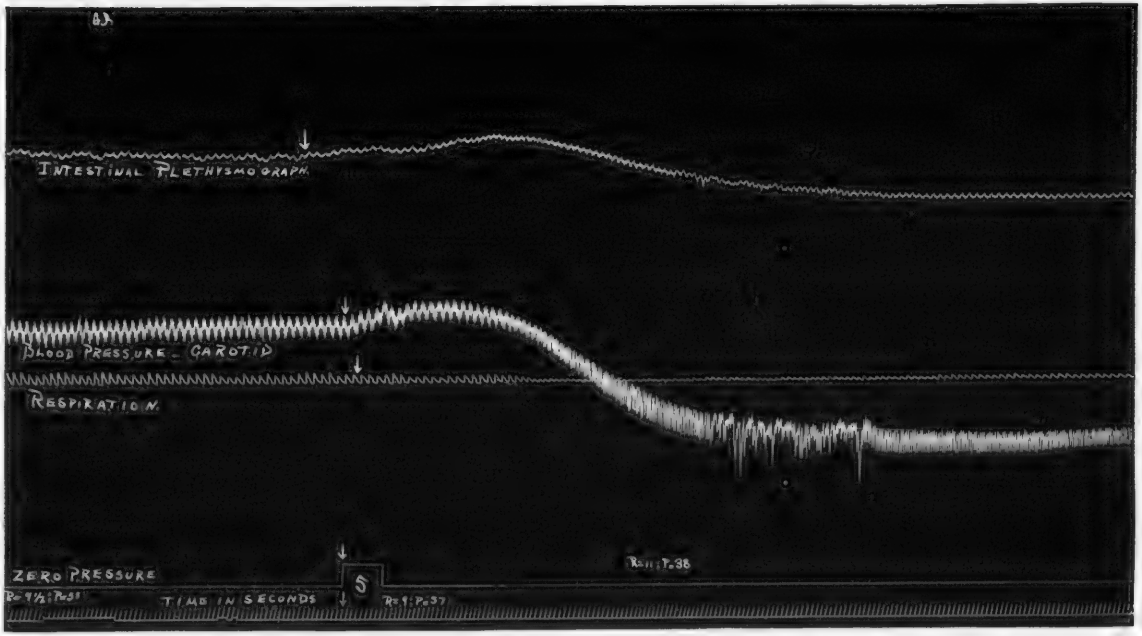


FIG. 2.

Brown and Loevenhart Effect of Hematin.

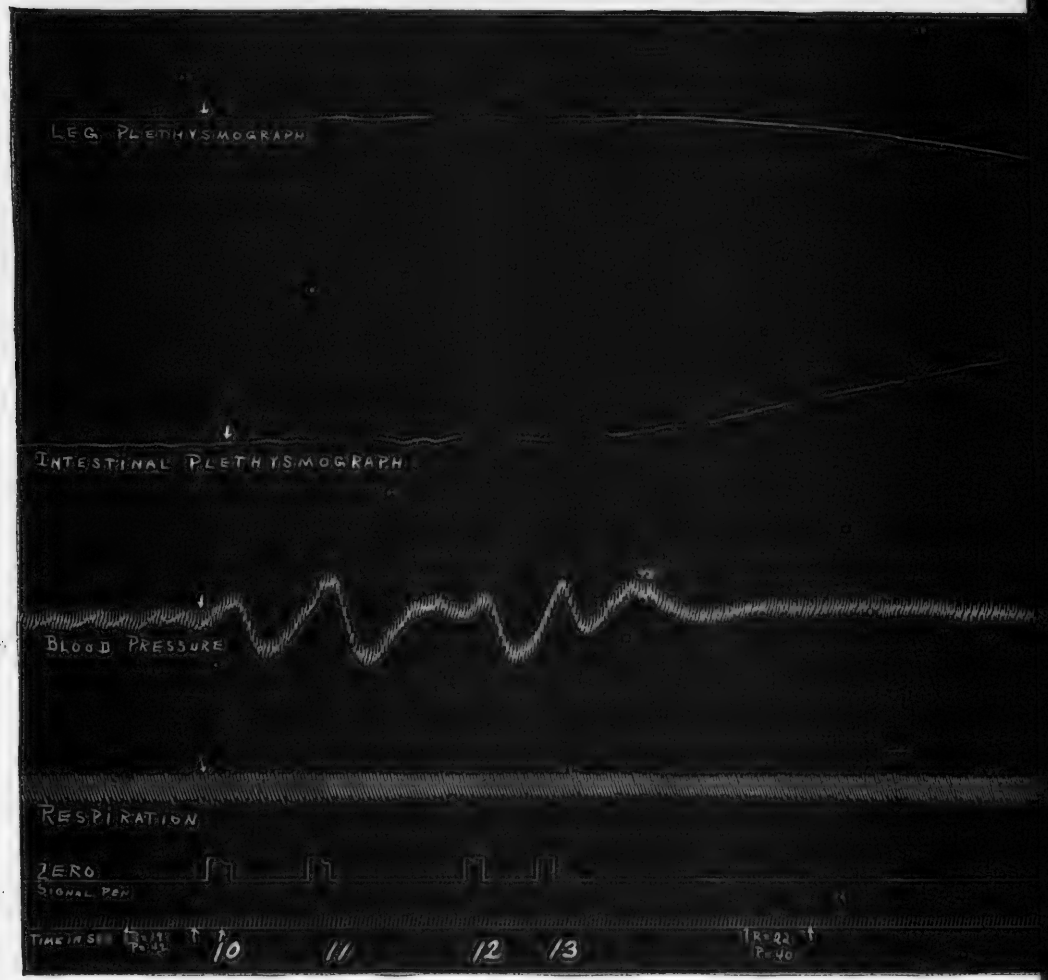

LEG PLETHYSMOGRAPH
INTESTINAL PLETHYSMOGRAPH
BLOOD PRESSURE
RESPIRATION
ZERO
SIGNAL POS
TIME IN SE
10
11
12
13

FIG. 3.

XVIII.

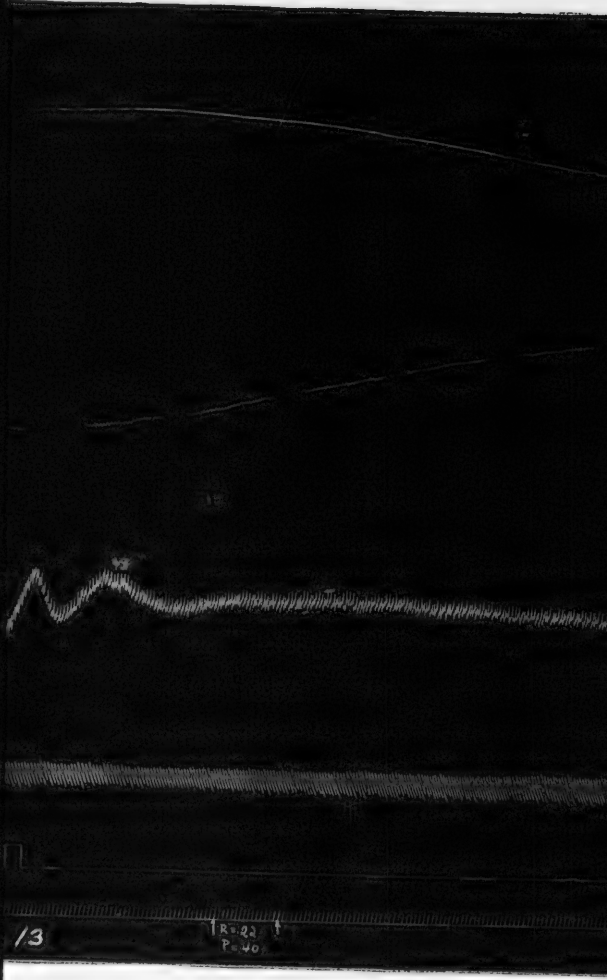


FIG. 3.

PLATE 4.

(Brown and Loevenhart: Effect of Hematin.)

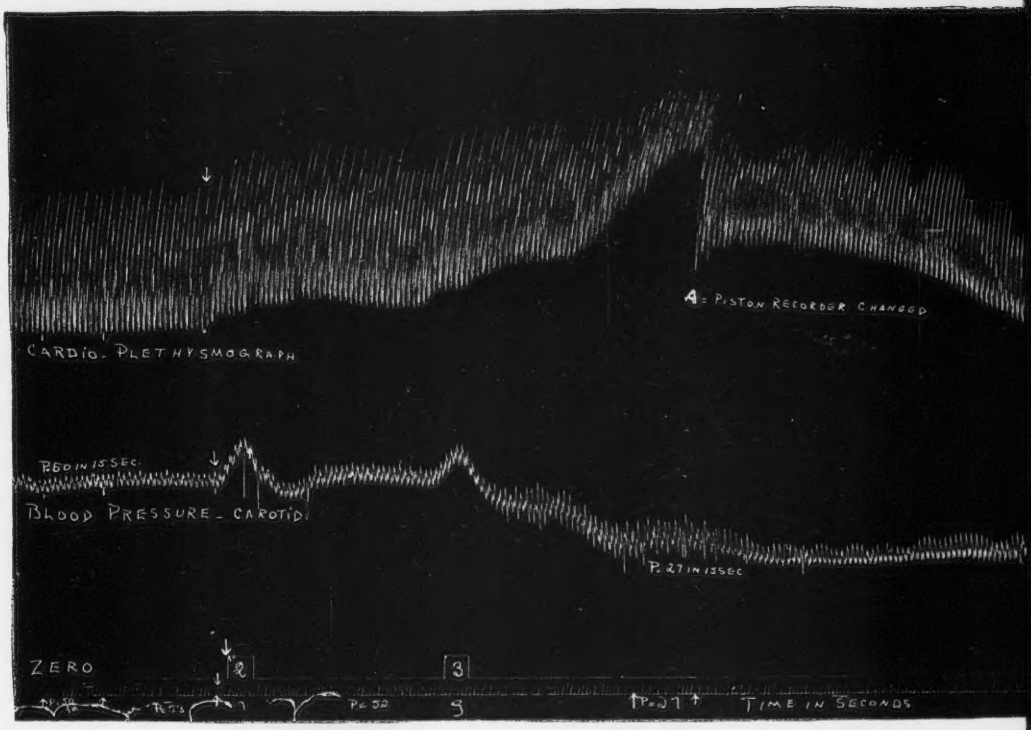

A = Piston Recorder Changed
Cardio-Plethysmograph
P. 60 in 15 sec.
Blood Pressure-Carotid
P. 27 in 15 sec.
Zero
2
3
Time in Seconds

FIG. 4.

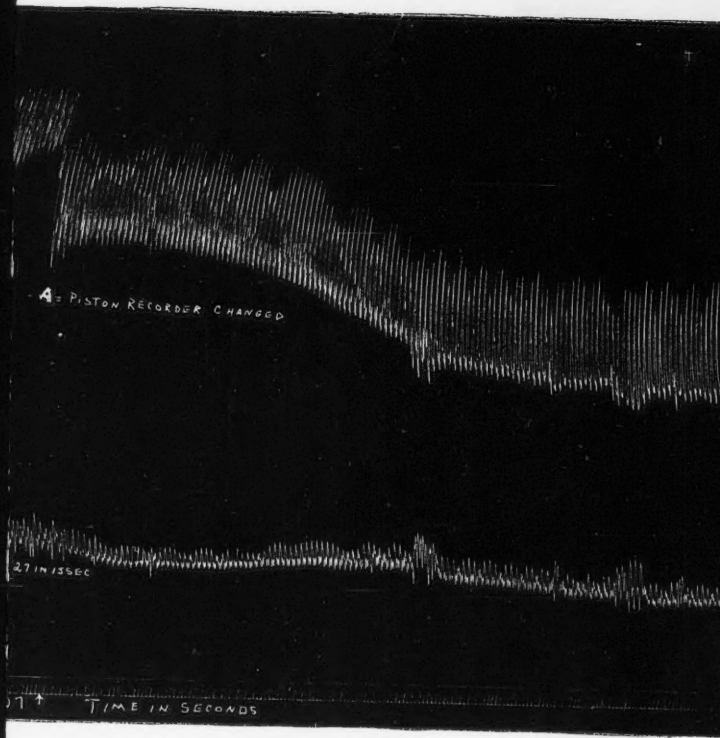
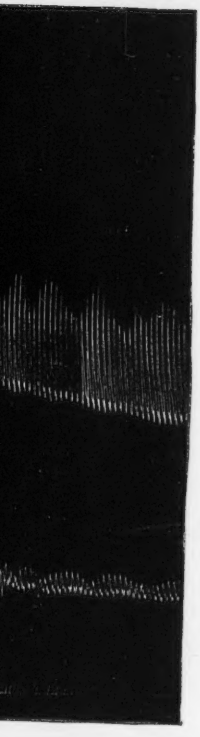


FIG. 4.

(Brown and Loevenh

PLATE 5.



rt : Effect of Hematin.)